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THE CHROMOSOMES OF HUMAN SPERMATOCYTES

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University of Cincinnati

FOUR PLATES

An explanation of the remarkable difference in the conclusions of von Winiwarter on the one hand and those of Guyer and Montgomery on the other, concerning the chromosomes in human spermatogenesis, has been sought in the fact that while von Winiwarter based his opinion on a study of the germ cells of white men, Guyer and Montgomery studied negro material (Guyer, '14, Morgan, '14). That there should be a difference in the number and behavior of the chromosomes in two races of animals does not strike one as an utter impossibility, but that the black race should possess 22 and 24 chromosomes, in the male and female respectively, while the white race should have approximately twice that number, namely, 47 in the male and 48 in the female, is rather astonishing to say the least; and certainly invites further investigation.

I have had opportunity for some years past to study human chromosomes both in the germ cycle and in embryos. In the fall of 1915, however, I succeeded in securing specimens of both white and negro testes, preserved within two hours after death, which on examination proved extremely favorable for a comparative study of the chromosomes of the two races.

The white material was obtained from a healthy white man, 37 years of age, who died from a stab wound (Cincinnati General Hospital Case No. 5749). The negro material was secured from a medical case (Cincinnati General Hospital Case No. 3463). Microscopic examination and comparison with other negro testes in my possession showed an active and healthy proliferation of germ cells that appeared to be entirely normal. For this

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and other material I am greatly indebted to Dr. Paul G. Woolley Director of the Pathologic Institute of the Cincinnati General Hospital, to whom my thanks are due.

The material was fixed in a modification of Bouin's solution (similar to Allen's B-15, used with and without chromic acid and urea) with excellent results. Sections were cut 7 micra in thickness and stained in two ways, namely, Schwaardemaker's safranin and Lichtgrün, and iron alum haematoxylin. These two methods are excellent checks on each other. Of the two probably safranin-lichtgrün is the more valuable for counting chromosomes and for differentiating between chromosomes and plasmosomes.

I. OBSERVATIONS

The most favorable period in the germ cycle in which to study the chromosomes proved to be the dividing stages of primary spermatocytes and for this reason considerable attention was devoted to this stage. As in most mammalian material the two principal difficulties in a study of this kind are, first, the fact that only rarely is a metaphase plate formed in which all of the chromosomes lie in one plane, and second, a tendency for the chromosomes to adhere in clumps. Generally the chromosomes begin dividing before an equatorial plate stage is reached and one must be very careful in making counts not to omit chromosomes lying above or below the main group. Side and oblique views and, best of all, early anaphase stages are valuable in checking up polar-view observations. In cases selected for careful study only cells whose chromosome groups were entirely included in the thickness of the section were used, except as noted below.

Figures 11, 12, 13, 14, 15 and 16 are drawings of primary spermatocyte chromosomes at what is approximately the metaphase stage of division. In figures 11, 12, 15 and 16 no division has occurred, and 12 obviously bivalent chromosomes may be counted. In figures 13 and 14 one chromosome in each group has divided and the products of this division are marked a_1 and a_2. All of these drawings were made from white material.

Figures 17, 18, 19 and 20 represent the corresponding stage in the negro. A careful study has revealed no essential differences in the two races. In figure 20 one of the chromosomes has divided into a_1 and a_2. The justification for this interpretation may not be entirely clear from the figure, but in the actual section the chromosomes a_1 and a_2 lie respectively considerably above and below the level of the remaining chromosomes. In figures 18 and 19 the m chromosome has divided into m_1 and m_2. In figure 17, 12 undivided chromosomes may be counted.

The chromosomes at this stage in either race show more or less constant differences in form and size. The most constant and conspicuous examples of this are seen in the large distinctly bivalent body, the components of which are unequal in size (XY, figs. 13, 14, 16, 17 and 18), and a small bivalent body m, appearing as a short constricted rod with rounded ends as in figures 12, 13, 14 and 15, etc., or as two spherules connected by a slender strand as in figures 17 and 20. As has been noted above, in figures 18 and 19 it has divided (m_1 and m_2).

Turning now to earlier stages, I have found abundant instances of dividing spermatogonia, but very few favorable for accurate counting. Figure 1 is a drawing of spermatogonial late prophase (white) in which 24 rod-shaped chromosomes may be counted. Figure 2 represents the same stage in the negro and likewise contains 24 chromosomes. The latter figure was drawn from two sections. I have not found it possible to identify in these groups the X and Y chromosomes. A pair of very small chromosomes may be seen in either plate which probably represent the constituents of the bivalent m chromosome of the primary spermatocyte.

The growth period is characterized by a very distinct bipartite chromatin nucleolus closely applied to the nuclear membrane and staining very intensely with the basic dye (figs. 4, 5, 6 and 7). Its size varies at different times in the growth period. Thus in the early part of the period it is very difficult to distinguish it in the tangled chromatin threads, but as growth proceeds it becomes more and more prominent until finally it is the most sharply defined object in the nucleus. Figure 5, x, y, shows it beginning to be recognizable. Figures 6 and 7 show it well developed. Its history and development are the same in both races. Figures 5 and 6 are from white material and figure 7 from the negro. Figure 4 gives an idea of its variations in form and size. Occasionally its constituents form two separate bodies. A large plasmosome, P, is also present at this time but it may be readily distinguished from the chromatin nucleolus both by its spherical form and the fact that it takes the acid stain. The chromatin nucleolus is distinctly bivalent and the components are unequal in size.

Figures 8, 9 and 10 illustrate some of the principal changes occurring in the prophase. In figure 8 the chromatin nucleolus (x, y) is rather sharply defined while the remaining chromosomes are ragged in outline and stain weakly. This section does not include the entire nucleus, a small part of which lay in the adjacent section. In some cases the distinctly bivalent character of these chromosomes may be seen. A circle, straight and twisted V's, and thick constricted rods may be seen. Including the chromatin nucleus 12 bodies may be counted in this section; although it is incomplete—the cut ends of some of the chromosomes lying in the next section.

Figure 9 shows the entire nucleus of a slightly later stage. The chromosomes have become more dense and deeply staining. Here again 12 bodies may be counted, as is also the case in figure 10 which is a still later stage. The dotted outline in this figure represents the outline of nuclear membrane which is disappearing. The problem of how the process of reduction has been brought about will not be entered into at this time.

From the facts discussed up to this point the following general conclusions are indicated:

1. The chromatin nucleolus of the rest stage in both the white and black race is formed by the union of two unequal spermatogonial chromosomes and maintains its identity throughout the entire period intervening between the telophase of the last spermatogonial division and the prophase of the primary spermatocyte.

2. The remaining 22 spermatogonial chromosomes lose their distinctness of outline in the early part of the growth period to emerge later as 11 bivalent chromosomes in the prophases of the primary spermatocyte.

Figures 21, 22 and 23 represent side views of the primary spermatocyte division in which at either end of the spindle are seen two unequal chromosomes very closely placed if not actually united (X_1 Y_1, X_2 Y_2) which I believe are the products of the divided XY of the preceding figures. In these cases each element of the XY has divided longitudinally considerably in advance of the division of the remaining 11 chromosomes. The early division of the XY is not an invariable rule as may be seen from figure 25 in which one of the other chromosomes has divided into b_1 and b_2 before the others. In other cases as in the anaphase pictured in figure 27 it is impossible to pick out the XY or its division products. In figure 26 two chromosomes are shown dividing in advance into c_1 c_2 and d_1, d_2. It may be that in this case X and Y elements became separated before dividing or it may be that two of the ordinary chromosomes have divided. Figure 28 is an anaphase in which the XY moieties can be distinctly seen well in advance of the other chromosomes. Figures 21, 22, 26 and 28 are from negro testes; figures 23, 24, 25 and 27 from white.

Figure 29 (white) shows an oblique view of an anaphase in which every chromosome has divided. The dotted line *ab* separates the two daughter groups each of which contains 12 chromosomes. In this case the *m* chromosome is the last to divide. The products of XY are shown at XY_1, and XY_2. Figure 30 shows the same stage in the negro. Here likewise the dotted line *ab* is the line of demarcation between the two daughter groups each containing 12 chromosomes. In the telophase stage of figure 31, it is possible to count 12 chromosomes at the left pole—the right pole being not entirely clear owing to fusion of the chromosomes. In figure 32 a large plasmosome (P) is seen near one pole. Without proper precautions in staining this body might be mistaken for a chromosome.

There is a well marked interkinesis stage as has been noted by Montgomery and von Winiwarter. Figures 33, 34, 35 and 36 represent typical appearances at this stage. The nucleus exhibits several chromatin nucleoli of various sizes. The fact that two of larger ones (figs. 33, 34, x_1 y_1) are much of the same shape as the chromatin nucleolus of the primary spermatocyte has led me to believe that they are the products of the divided XY. The appearance varies in different regions of the seminal tubules. In figure 35 and 36 for example the resemblance between the chromatin nucleoli of this stage and the earlier primary spermatocyte is not so clear. This is due, I believe, to the fact that the appearance of the interkinesis nucleus varies at different periods. In the early part of the period before the chromosomes have gone completely into the resting state one finds a larger number of chromatin masses than in later stages. Therefore if the resting stage of the primary spermatocyte be any kind of a guide, the stage at which the bivalent chromatin nucleolus shows most distinctly is the stage at which the ordinary chromosomes have practically disappeared, as in figures 33 and 34 which show a remarkable resemblance to figure 7.

Figures 37, 38 and 39 are late prophases or early anaphases of the secondary spermatocyte in which 12 chromosomes including an XY element may be seen. In figure 39 the XY is almost separated into two parts. I have not as yet been able to work out in detail the subsequent division of the secondary spermatocyte. I can only point out what seems to be the obvious inference that in this division the XY element breaks at the point of union, the X and Y passing undivided to opposite poles of the spindle, while the remaining chromosomes all divide. The result would be that one-half of the total number of spermatids would receive 11 chromosomes + X and the other half 11 chromosomes + Y.

II. DISCUSSION

In 1910 Guyer published an account of the chromosomes in human spermatogenesis based on the study of a negro testis, in which he described and figures 22 chromosomes in the spermatogonial metaphase.

> In a few instances two, apparently the two accessory chromosomes, were seen considerably to one side of the main mass of chromosomes, surrounded by a small clear court of cytoplasm. Twelve chromosomes appear for division in the primary spermatocyte, of which ten are evidently bivalent and two accessories. The two accessory chromosomes pass undivided to one pole of the spindle considerably in advance of the other chromosomes with the result that half of the daughter cells in this division receive twelve, and half only ten univalent chromosomes . . . . The ten univalent chromosomes which passed to the one secondary spermatocyte unite again in pairs, at least in the majority of cases, to form five bivalent chromosomes which appear at the equator of the spindle when the cell is ready for division. The division here presumably is an equation and not a second reduction division, judging from the size, shape and general appearance of the resulting daughter chromosomes. . . . . There is some slight evidence that the secondary spermatocytes may occasionally divide with these chromosomes in their original condition of univalence. Ten of the twelve chromosomes which passed to the other pole of the spindle in the primary spermatocyte behave in precisely the same way. . . . . The two accessory chromosomes come to the equator of the spindle in the secondary spermatocyte with the five bivalent, thus making seven in all. Each accessory now divides so that the resulting spermatids each receive seven chromosomes; that is five bivalents plus two accessory, or the equivalent of twelve univalent chromosomes . . . . Of the total number of spermatids, half have in all probability received ten and half twelve (10 plus 2) univalent chromosomes (pp. 230–231).

Montgomery in 1912 based his study of human spermatogenesis in part on portions of the same material studied by Guyer and in part on testes of another negro. In his paper he does not figure spermatogonial plates. In the late prophases of the primary spermatocytes he found 12 chromosomes. "Ten of these must be gemini or bivalent chromosomes judging by their late history and by analogy with other species. . . . The two remaining elements are the univalent allosomes, but in these late prophases it is practically impossible to say which two these are" (p. 5). It may be noted that Montgomery's allosomes correspond to Guyer's accessory chromosomes (i.e., *XX*) and to what I call the *X* and *Y* chromosomes. Montgomery believed, however, that there is considerable variation in the distribution of the *X* and *Y* chromosomes. In his own words,

> We may now summarize the allosome behavior in the primary spermatocytes with respect to their distribution to the secondary spermatocytes and from this infer their distribution to the spermatids, using

> the letters *D* and *d* to denote the larger and smaller allosome respectively. In so doing we should recall that each allosome divides only once in the course of the two maturation mitoses, and undergoes one transport (reductional) without division.
>
> *Condition A, 59 cases.* Both *D* and *d* at one spindle pole (of the first spermatocyte). Both would then go to one secondary spermatocyte and in that one divide equationally. 118 spermatids would then each contain $\frac{1}{2} D$ and $\frac{1}{2} d$, while 118 would receive no part of these. This is the most usual condition and the one discovered by Guyer.
>
> *Condition B, 5 cases.* *D* at one spindle pole and *d* at the opposite pole. One secondary spermatocyte would receive *D* entire, and the other *d* entire. These dividing in the secondary spermatocytes would result in 10 spermatids each with $\frac{1}{2} D$ and 10 each with $\frac{1}{2} d$.
>
> *Condition C, 10 cases.* *D* at one spindle pole, $\frac{1}{2} d$ at that pole and $\frac{1}{2} d$ at the opposite pole. Half the secondary spermatocytes would receive only $\frac{1}{2} d$, which does not divide again, consequently from this line would result 10 spermatids with $\frac{1}{2} d$ and 10 with no allosome. The remaining secondary spermatocytes would receive *D* and $\frac{1}{2} d$; the former would divide in them but not in the latter, and there would result 10 spermatids with $\frac{1}{2} D$ and 10 with $\frac{1}{2} D$ and $\frac{1}{2} d$.
>
> *Condition D, 5 cases.* *D* probably dividing at the equator (for it is absent at the poles), *d* at one spindle pole. Half the secondary spermatocytes would receive *d* and $\frac{1}{2} D$; in them *d* would divide but not $\frac{1}{2} D$, and there would be formed 5 spermatids with $\frac{1}{2} d$, and 5 with $\frac{1}{2} d$ and $\frac{1}{2} D$. The other secondary spermatocytes would receive only $\frac{1}{2} D$, which would not divide in them, consequently 5 spermatids would receive $\frac{1}{2} D$ and 5 would receive none.
>
> *Condition E, 3 cases.* Both *D* and *d* dividing in the equator. Every secondary spermatocyte would then receive $\frac{1}{2} D$ and $\frac{1}{2} d$, and these would not divide again. It would then be a matter of chance how these allosomes became distributed to the spermatids. There might be: either 6 spermatids with $\frac{1}{2} D$ and 6 with $\frac{1}{2} d$; or 6 spermatids with $\frac{1}{2} D$ and $\frac{1}{2} d$, and 6 spermatids with no allosomes. . . . That is (omitting condition E) 42.09 per cent of the spermatids contain 2 allosomes, the same number contain no allosomes, and 15.82 per cent contain one allosome.
>
> There are then in man certainly four classes of spermatozoa with regard to their allosome content, and possibly five or six. Scarcely any of the spermatozoa examined show abnormalities and no degenerating ones were found, therefore there is no reason to believe that all but certain classes of sperm degenerate or prove incapable of fertilization (p. 9 and 10).

Concerning another point Montgomery says (p. 12),

> Bardeleben held that a second reduction occurs in the secondary spermatocytes resulting in approximately a quarter of the normal number in the spermatoids. Guyer found about the same result, concluding of the secondary spermatocytes that "half of them show five and

> the remainder seven chromosomes." . . . . I have seen no evidence of any kind of such a pairing of chromosomes in the second spermatocytes, neither in my own material nor in that received by Guyer, although I have examined fully two hundred division stages of these cells. Of decisive value are such cases, of which several are figured by me, where all the chromosomes can be distinctly seen on lateral views of spindles of the second maturation. The only explanation I can offer for this conflict of opinion is that Bardeleben and Guyer either employed too intense staining of their sections, or else studied cells in which the chromosomes had been greatly swelled by fixation and hence not clearly distinguishable.

I might say in passing that my own observations lead me to agree with Montgomery's explanation of the so-called second reduction.

Von Winiwarter ('12) used for his study of human spermatogenesis sections of testis of four men (presumably white) aged 21, 23, 25 and 41 years, all which seemed to be free of any venereal taint or infection. He states that the material was well fixed and offered abundant opportunity to study chromosomes under favorable conditions. He does not state how soon after death the tissues were fixed in the weak Flemming, modified by Meves, which he used.

As to the number of chromosomes in the spermatogonia he states (p. 130), "In 32 plates I count 47 chromosomes 29 times, 46 chromosomes 2 times and 49 once." A careful study convinces him that 47 is the bivalent number in man. It may be noted that he also found occasional plates containing 65, 70, 86, etc., up to 100 and 150. These he considers in the nature of exceptions common to the germ cycle of all animals.

In the nucleolus of the resting stage, he finds an acid staining nucleolus (plasmosome) and an elongated bacilliform, basic staining body, the chromatin nucleolus or accessory chromosome, which lies close to the nuclear membrane. In the prophases of the primary spermatocyte the accessory chromosome shows no evidence of duality in structure. The remaining chromosomes showed a pronounced bivalent appearance. In the majority of cases he finds 24 chromosomes, only one of which, the accessory, fails to divide in the ensuing division of the first spermatocyte.

A second rest stage or interkinesis follows. In the prophases of the second spermatocyte all of the chromosomes appear biscuit-shaped. Of 25 plates examined 24 chromosomes were found 15 times, and 23 chromosomes 10 times. All of the chromosomes divide in this division giving therefore two kinds of spermatids; one half the total number containing 23 and the other half 24 chromosomes.

Von Winiwarter further found in the ovary of a four month foetus three cases of dividing cells in which 48 chromosomes could be counted, thus partially at least realizing the expectation indicated by his study of the testis.

> L'espèce humaine mâle comporte 47 chromosomes somatique, l'espèce femelle 48. La fécondation d'un oeuf, contenant 24 chromosomes, par un spermatozoïde à 24 chromosomes aboutit au chiffre 48 c'est-à-dire à une femelle, la fécondation par un spermatozoïde à 23 chromosomes au chiffre 47, à un mâle (p. 145).

It will be readily understood from the foregoing that while my findings incline more toward agreement with those of Guyer and Montgomery than those of von Winiwarter, there are a number of points of difference.

In the first place I believe that the spermatogonia contain 24 chromosomes rather than 22. The determination of the number based on counts of spermatogonial plates alone is not a simple matter in such difficult material and a difference of 2 in 24 is not a great deal. As a matter of fact I have never counted less than 24 in material and in a few cases 25 and 26. However, the evidence from the study of the growth period and spermatocyte division figures taken in conjunction with the facts of spermatogenesis in other animals leaves no doubt in my mind that the bivalent number in the material studied by me is 24 rather than 22 or 47.

In the rest stage there is a well defined chromatin body consisting of two unequal parts occasionally separated. Montgomery (in figs. 2, 3, 4, 5, 6 of his paper), pictures this body sometimes with the constituents united and sometimes separated. I have seen both conditions in my material, but more often with the parts united. Guyer's figures are indecisive on this point since they do not show the plasmosome. Von Winiwarter's claim that this body is single, i.e., unpaired, I can not confirm.

This bipartite chromatin body seems to be an unequal pair of idiochromosomes which can be traced from the telophase of the last spermatogonial division through the rest stage to the prophase of the primary spermatocyte (figs. 5, 6, 7, 8, 9 and 10). In the prophases and in the later metaphases 12 presumably bivalent chromosomes may be counted, counting the *XY* pair as one. Guyer and Montgomery also find twelve chromosomes at this time but they consider the *X* and *Y* as single univalent chromosomes forming two of the twelve.

I have already considered Guyer's and Montgomery's interpretation of the distribution of the two idiochromosomes. I too have found side views which support Guyer's view that the two (accessory chromosomes) pass undivided to one pole of the primary spermatocyte spindle. I also find division figures illustrating the various possibilities considered by Montgomery; but I am inclined to believe that *X* and *Y* *divide* in this division both from the undeniable evidence of side views (figs. 21, 22, 23 and 28) and, what seems to me is more important, from the fact that I always found that daughter groups in anaphase stages contain an equal number of chromosomes, namely 12, counting the two parts of the divided *XY* as one (figs. 29 and 30). It may be, of course, that an occasional variation in the distribution of this body does occur, but the evidence from my material points to its division in the primary spermatocyte as the usual method of distribution. It may be noted that Montgomery also found this condition in a number of cases ("Condition E").

I can confirm Montgomery and von Winiwarter in the presence of an interkinesis stage. The occurrence of chromatin structures in the nucleus at this time comparable to the chromatin nucleus of the primary spermatocyte also points to the division of the *XY* in the first maturation mitosis.

In the secondary spermatocyte division I find 12 chromosomes, one of which in most cases can be identified as *XY* by its peculiar shape. I have not been able to determine by direct observation of anaphases what happens in this division. Everything would point, however, to a division of all the chromosomes; *X* and *Y* being separated and passing to opposite poles.

I am utterly unable to confirm von Winiwarter's statements and figures as to the number of chromosomes. I have occasionally found giant spermatogonia in which about 48 chromosomes could be counted, but these from their rarity obviously cannot represent the normal condition. Figure 3 shows a metaphase plate of one of these giant cells. Further, the difference in results cannot be due to difference between European and American, because it happens that the subject whichfurnished the white human testis on which most of my observations are based was a native of Germany. It would appear from the most recent evidence of other investigators that conditions described by von Winiwarter constitute an exception the explanation of which is not yet clear.

III. SUMMARY

1. The metaphase plates of human spermatogonia contain 24 straight or bent rod-shaped chromosomes, two of which are presumably idiochromosomes forming an *XY* pair.

2. Throughout the growth period of the primary spermatocyte, the idiochromosomes persist as a basic staining bipartite body whose components are sometimes separated in which case there are two chromatin nucleoli in the nucleus instead of a single paired one.

3. In the prophase of the primary spermatocyte 12 bivalent chromosomes appear, one of which is the *XY* pair.

4. The *XY* pair divides longitudinally in the primary spermatocyte division. The remaining 11 bivalent chromosomes also divide in this division but whether quantitatively or qualitatively was not determined.

5. An interkinesis stage follows in which the nucleus contains a double chromatin body resembling the chromatin nucleolus of the first spermatocyte. It is suggested that this body is one half of the longitudinally split *XY* pair of idiochromosomes which persist throughout the interkinesis stage.

6. The second spermatocyte metaphase plates contain 12 chromosomes, one of which can be recognized as a half of the split *XY* pair. In the division it is assumed that the *X* and *Y* constituents pass undivided to opposite poles while the remaining 11 chromosomes all divide. The result would be that one half the spermatids receive 11 ordinary chromosomes plus X and the other half 11 plus *Y*.

7. The number and behavior of the chromosomes in the spermatogenesis of the white and negro races of man is the same in the material studied.

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EXPLANATION OF PLATES

All figures are table level drawings outlined with the camera lucida at the magnification produced by 1.5 mm. Zeiss objective and 18 compensating ocular. Details were drawn in with the same objective and 12 compensating ocular. The monobjective binocular microscope was found to be very useful in verifying the findings of the ordinary compound microscope. Owing to differences in angle from which the chromosomes were drawn, there is some variation in size of individual chromosomes in different figures. The figures have been reproduced $\frac{1}{6}$ off.

ABBREVIATIONS

X, *Y*, the members of a pair of unequal idiochrosomes.

x, *y*, the members of bipartite chromatin nucleolus of the growth period of the first spermatocyte, and also of the interkinesis nucleus.

m, small bivalent chromosome.

p, the plasmosome of the primary spermatocyte.

Other abbreviations explained below.

PLATE 1

EXPLANATION OF FIGURES

1 Spermatogonium, late prophase, white, showing 24 chromosomes.

2 Spermatogonium, late prophase, negro, showing 24 chromosomes.

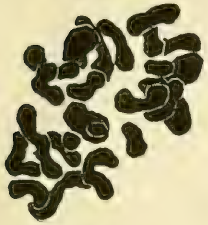
The difference in size in the two figures is, I believe, without special significance. Figure 1 is from a Safranin-Lichtgrun preparation and figure 2 from an iron-alum-haematoxylin slide. The latter is reconstructed from two adjacent sections.

3 Large abnormal spermatogonial metaphase, white, showing approximately 48 chrómosomes.

4 Examples of various types of chromosome nucleolus.

5 and 6 Primary spermatocyte, growth period, white.

7 Primary spermatocyte, nucleus, growth period, negro.



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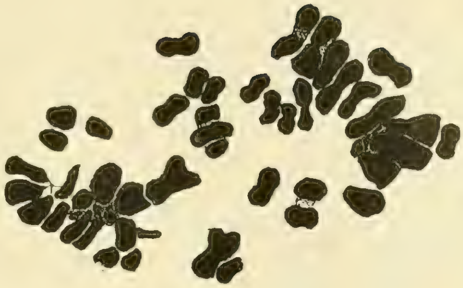

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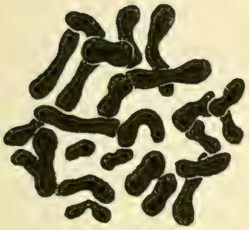

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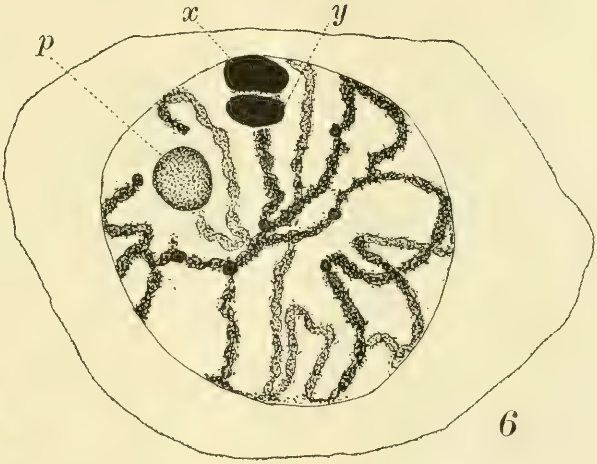

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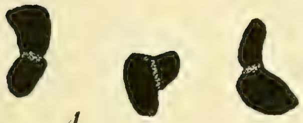

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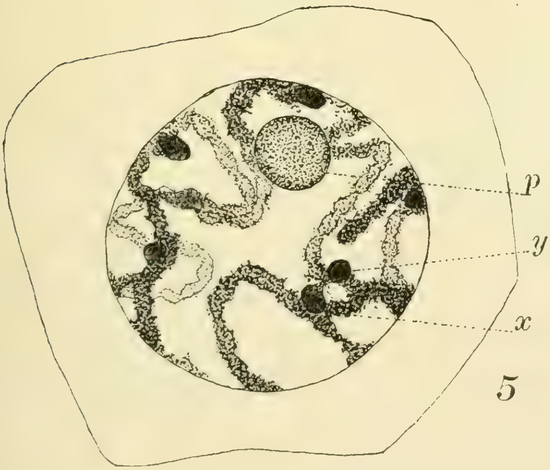

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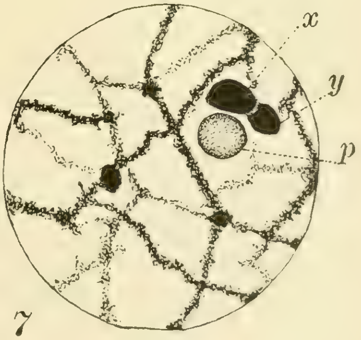


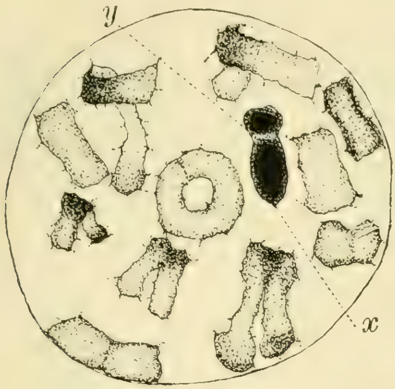
7

PLATE 2

EXPLANATION OF FIGURES

8, 9 and 10 Primary spermatocyte, prophase, white.

11, 12, 13, 14, 15, 16 Primary spermatocyte, metaphase plates, white, showing 12 chromosomes. In figures 13 and 14 chromosomes a_1 and a_2 are the halves of a divided chromosome. In figure 16 the m chromosome is seen from the end and therefore presents a round profile.



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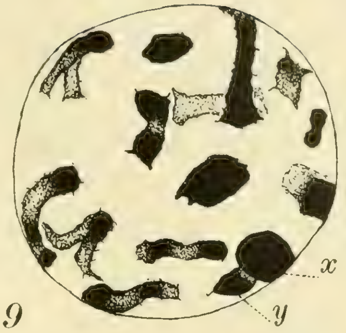

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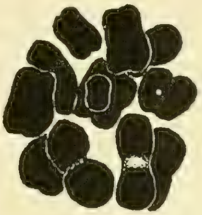

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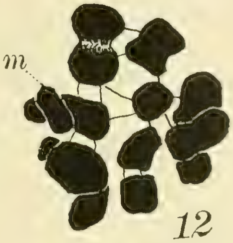

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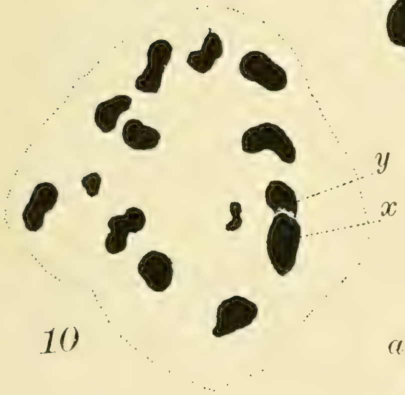

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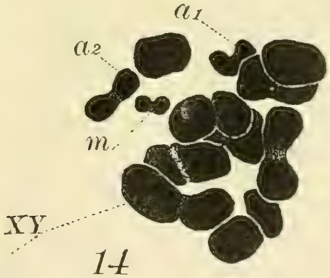

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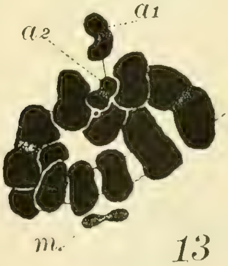

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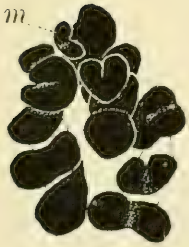

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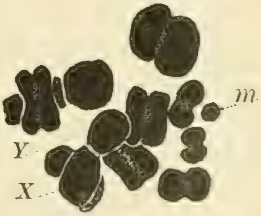

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PLATE 3

EXPLANATION OF FIGURES

17, 18, 19 and 20 Primary spermatocyte, metaphase plate, negro, showing 12 chromosomes. In figure 20 a_1 and a_2 are the halves of a divided chromosome. In figures 18 and 19 m has divided into m_1 and m_2.

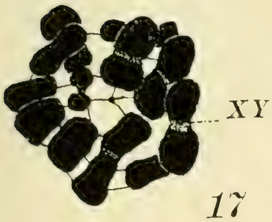
21 and 22 Primary spermatocyte, metaphase side view, showing XY elements divided longitudinally before the other chromosomes; negro.

23, 24 and 25 Primary spermatocyte, metaphase side view. In figure 23, the XY element has divided. In figure 24 none of the chromosomes have divided. e is a large bivalent chromosome out of plane with the majority of the chromosomes. In figure 25 b_1 and b_2 are parts of a divided chromosome probably not the XY; white.

26 Primary spermatocyte metaphase side view showing at c_1, c_2, d_1 and d_2 the halves of two chromosomes already dividing. These may or may not be the products of the XY chromosome; negro.

27 Primary spermatocyte anaphase showing most of the chromosomes divided; white.

28 Primary spermatocyte anaphase showing the divided XY element at either pole and away from the other chromosomes; negro.



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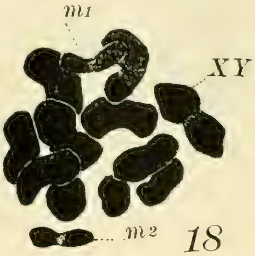

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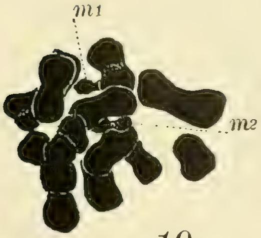

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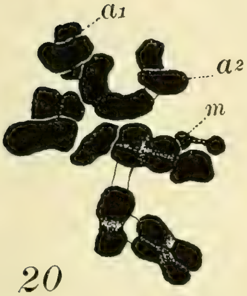

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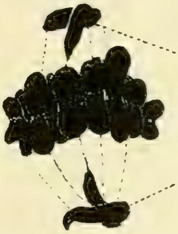

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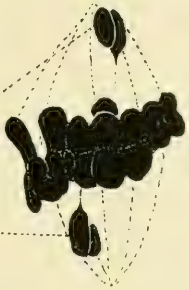

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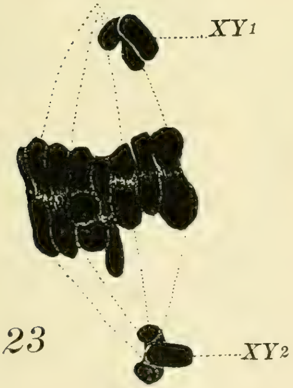

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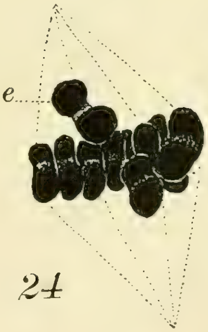

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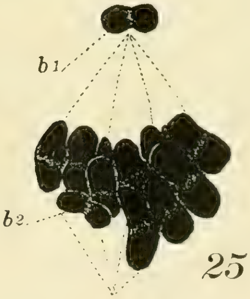

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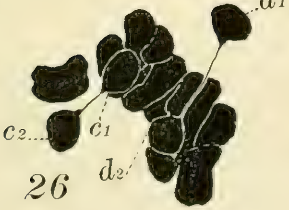

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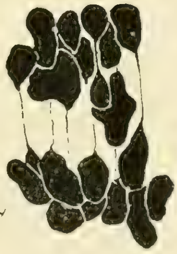

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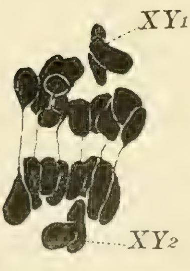

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PLATE 4

EXPLANATION OF FIGURES

29 Primary spermatocyte anaphase, oblique view showing 12 chromosomes at either pole. The dotted line *a–b* separates the two groups; the *m* chromosome is the last to divide; white.

30. Same stage as figure 29; negro.

31 and 32 Primary spermatocyte telophase. In figure 31 12 chromosomes may be counted at the left pole of the spindle. The chromosomes at the other were fused and indistinct.

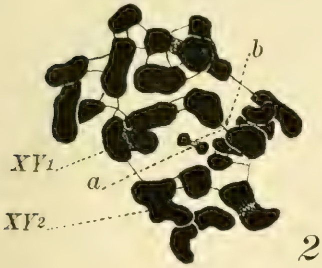
In figure 32 a large plasmosome may be seen near the lower pole of the spindle; white.

33, 34, 35 Characteristic interkinesis stages, showing chromatin nucleoli which suggest the *X* and *Y* elements. In figure 35 the *X* element lies above the *Y* element which is not shown; white.

36 Interkinesis stage showing 2 chromative nucleoli which very likely are the separated *X* and *Y;* negro.

37 Secondary spermatocyte metaphase plate showing 12 chromosomes; white.

38 and 39, Secondary spermatocyte plate, showing 12 chromosomes; negro.



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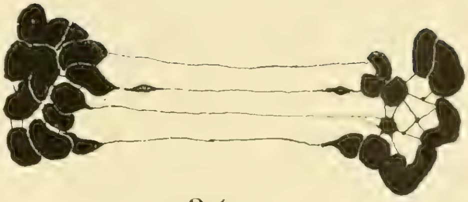

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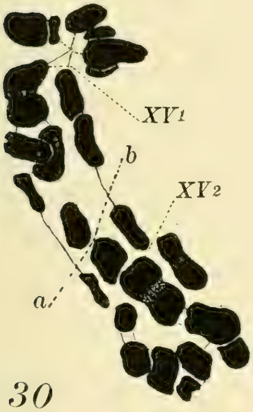

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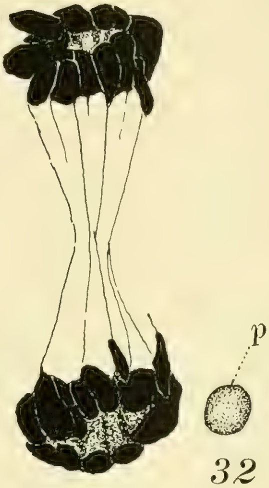

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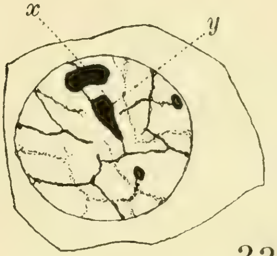

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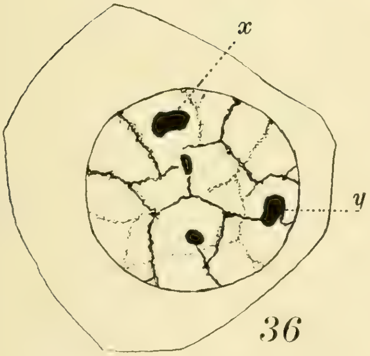

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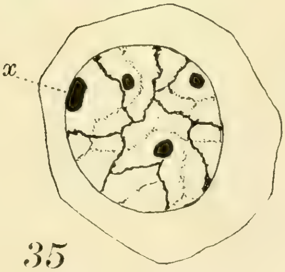

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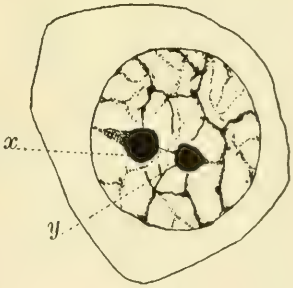

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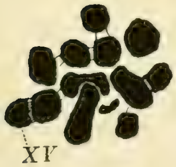

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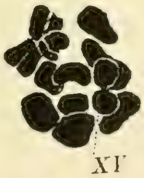

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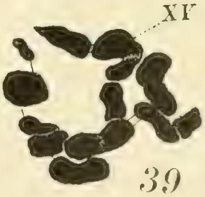

39

CHANGES IN THE COMPOSITION OF THE ENTIRE BODY OF THE ALBINO RAT DURING THE LIFE SPAN

S. HATAI

The Wistar Institute of Anatomy and Biology

ONE CHART

The present investigation is intended to supplement a series of anatomical data which have already been recorded on the growth of the albino rat during its life span. As a first step in the chemical investigation of the body as a whole, the changes in the composition of the entire rat at different ages in respect to its four main components; water, protein, fat and ash, have been determined and the results are presented in the following pages.

In making the present analysis, the body was dried in an oven at the temperature of 95°C. and the dried residue thus obtained was subjected to a further analysis. Koch's ('13) investigation shows that the material which has been dried by heat yields slightly greater amounts of water soluble materials than the fresh material which has been immediately placed in alcohol. Consequently the value of the water extractives given in this paper may be slightly high, nevertheless I feel that even if such a difference occurs it is negligible when the degree of individual variation is considered.

I may state also that the extraction by ether and alcohol was carried on until no more typical lipoid color was visible, even after three hours continuous treatment. In isolating the water soluble fraction from ether-alcohol extractives, I have followed the technique devised by Koch and Mann ('09).

The albino rats used for the present investigation were obtained from the colony of rats kept at The Wistar Institute and were in good health. In all cases the contents of the entire digestive tract were carefully removed before the body was dried for further analysis.

1. External bodily changes during the first six weeks of life of the albino rat

A brief description of the external bodily changes during the first six weeks of life may assist us to interpret some of the chemical changes which are in progress at this time. I shall therefore present a brief diary of this period.

a. Rats are born naked. The eyes are closed.

b. At the end of one week the skin shows a tinge of white due to a growth of minute hairs. The eyes are still closed. The body has more than doubled its initial weight. Milk is the exclusive diet.

c. At about fourteen or fifteen days of age the eyes open. The body weight is more than three times that at birth. The skin is covered with short hairs. Milk is still the exclusive diet.

d. At twenty-one days after birth the rats are running about the cage and occasionally eat bits of food present in the cage. Thus the diet is mainly milk and in small part other food stuffs. The hairs are long and the coat velvety.

e. From this period on the rats grow without showing any marked external alterations except the enormous growth of the testicles in the males at the age of four to five months.

We see from the foregoing that the greatest external changes take place within the first three weeks, that is during the period when the rats are feeding entirely on milk. It will be seen later that the greatest changes in composition also take place within this same brief period.

2. Composition of the stomach contents

As has been stated, milk is the exclusive diet of the rat for the first three weeks of life, the period during which the most important changes in the composition of the body take place. It is highly desirable therefore to obtain data on the chemical composition of the milk. Difficulty at once arises in obtaining directly a sufficient quantity of milk for investigation. I have therefore decided to follow a somewhat indirect method and to examine the stomach contents of young rats that are taking mother's milk exclusively. Although the composition of the stomach contents may not exactly represent that of normal milk, nevertheless by care in collecting it before it has been acted on by the gastric juice for any length of time, we may yet obtain some useful information. The following is the table showing the chemical composition of the stomach contents which had been carefully collected from the rats one to fourteen days old.

TABLE 1

Showing the composition of the stomach contents of the young rats which are taking mother's milk

WATER	SOLIDS	RESIDUE		FAT		ORGANIC EXTRACT	
per cent	*per cent*	*per cent of solids*	*per cent of moist weight*	*per cent of solids*	*per cent of moist weight*	*per cent of solids*	*per cent of moist weight*
54.42	45.58	22.11	10.11	69.13	31.57	5.31	2.43

SOLUBLE SALTS		FIXED SALTS		TOTAL ASH		ASH IN LIPOID FREE SOLIDS	ASH IN RESIDUE
per cent of solids	*per cent of moist weight*	*per cent of solids*	*per cent of moist weight*	*per cent of solids*	*per cent of moist weight*	*per cent*	*per cent*
2.00	0.93	1.45	0.68	3.50	1.61	11.08	6.22

I give also in table 2 the data on the chemical composition of the milk of several other mammals (compiled from the data given by König '03) which may be compared with that of the albino rat.

The comparison reveals the fact that the 'milk' of the rat is highly concentrated, and thus the content of the fat is remarkably high. Whether or not this high concentration of the milk was due to a rapid elimination of water from the stomach to the lower part of the digestive tract, cannot be determined, though I may remark in this connection that the absorption of water through the stomach wall is insignificant, and at the same time the gastric secretion must tend to increase the water. It appears therefore that this high concentration of the milk noted above, might after all indicate a normal condition of the fresh milk.

TABLE 2

Showing the data on the composition of the milk of several mammals compared with that of the stomach contents of the suckling albino rats

SPECIES	WATER	PERCENTAGE COMPOSITION OF SOLIDS			
		Protein	Fat	Lactose	Ash
	per cent				
Man	87.58	16.22	30.11	51.28	2.39
Cow	87.17	27.68	28.76	38.03	5.53
Cat	81.63	49.42	18.29	26.72	5.57
Dog	77.00	42.26	40.25	13.52	2.97
Rabbit	69.50	50.95	34.26	6.39	8.40
Rat (stomach contents)	54.42	22.11	69.13	5.31[1]	3.50

[1] Water extractives.

I shall discuss the content of fat later in connection with the content of fat in the entire body during the suckling period (p. 30). I may state here simply that the high fat content of rat's milk is easily understood when the other components are considered.

Another interesting fact which is to be noted is that the protein content of the milk in relation to the rate of body growth in the rat is quite harmonious with other mammals, as will be seen from the following table (after Abderhalden '99).

It appears from this agreement in relations that the stomach contents here analysed may fairly well represent the composition of the normal fresh milk.

TABLE 3

Showing the relations between the protein content of the total milk and the rate of body growth in several mammals

SPECIES	DAYS REQUIRED TO DOUBLE BIRTH WEIGHT	PROTEIN	ASH	SPECIES	DAYS REQUIRED TO DOUBLE BIRTH WEIGHT	PROTEIN	ASH
		per cent	*per cent*			*per cent*	*per cent*
Man	180	1.6	0.20	Pig	14	5.2	0.80
Horse	60	2.0	0.40	Cat	9½	7.0	1.02
Cow	47	3.5	0.70	Dog	9	7.4	1.33
Goat	22	3.7	0.78	Rat	6	10.1	1.61
Sheep	15	4.9	0.84	Rabbit	6	14.4	2.50

3. The growth of dry substance

In order to determine the amount of moisture in the body, the rats (after two weeks of age) were minced in the meat grinder. During the process of grinding a slight amount of blood and tissue is lost. However this loss is negligible in comparison with the entire body weight. Some water is also lost from evaporation, though it may be very slight, but as we are estimating the moisture in terms of the intact body weight, such loss will not disturb the results. The bodies of the smaller rats were dried directly after several incisions had been made on the body, as well as an opening into the skull. The bodies were dried in the oven at a temperature of 95° to 98°C. for one week. One week at such a temperature is more than sufficient to bring the material to a constant weight.

The weight of the dry substance during the first six weeks of life, together with that at 294 days, is given in table 4, and its graphic representation in chart 1.

TABLE 4

Showing the growth of dry substance during the life span of the albino rat

	AGE IN DAYS							
	Birth	7	15	22	28	35	42	294
Number of rats	43	7	9	5	3	3	3	2
Body grams	4.3	10.2	13.5	24.9	47.3	52.5	65.8	277.5
Water, percentage	87.2	79.8	72.9	70.6	69.6	70.6	69.4	65.3
Solids, grams	0.6	2.1	3.7	7.3	14.4	15.5	20.1	96.4
Solids, percentage	12.8	20.2	27.1	29.4	30.4	29.4	30.6	34.7

Chart 1 shows that the dry substance increases very rapidly, especially during the first two weeks of life. At the end of the third week the proportional amount of solids in the entire body (30 per cent) reaches almost the maximum (35 per cent). Thus after the end of the third week the increase is small compared with the increase which took place during the first three weeks, and indeed at the end of one year its increase is only slightly over 5 per cent more than that at the end of the third week.

This rapid increase of the dry substance is extremely interesting in view of the high value reached while the young are still nourished almost entirely by mother's milk. According to Donaldson ('06) the life span of the albino rat is about one-thirtieth of that of man. Taking man's normal span as ninety years and that of the rat as three years, we find that twenty-one days of rat age corresponds nearly to the end of the second year of childhood in man. Whether or not the dry substance of the human body approaches 90 per cent of the maximum at the end of the second year will be an interesting point to determine. Unfortunately this determination can not be made from the existing data. There are however in the literature data on other mammals, and I have compiled the following table from the data given by Aron ('15) to show the relative growth of the dry substance in several other mammals contrasted with such growth in the albino rat.

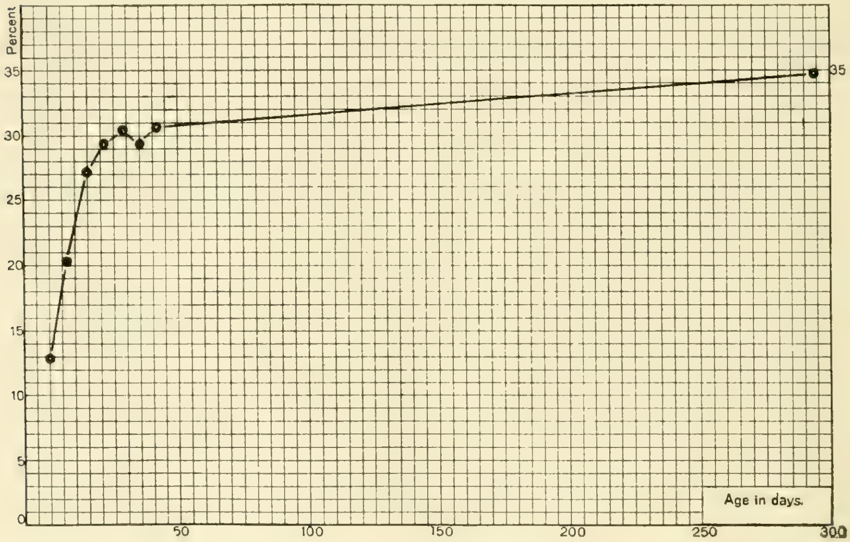

Chart 1 Showing the growth of the dry substance of the albino rat during its life span.

On account of the lack of necessary data we are unable to judge the growth rate of the dry substance in other mammals during their suckling period. Nevertheless we note from table 5 that the rats are born in the most immature condition, so far as the proportional weight of water and solids is concerned.

TABLE 5

Showing the relative growth of the dry substance in several mammals compared with that in the albino rat

SPECIES	RAT	MAN	CAT	DOG	PIG	RABBIT	MOUSE	GUINEA PIG
Age............	New born	5 months (fetal)				21 days (fetal)	Embryo	
Per cent of dry substance....	12.81	12.73				13.7	12.84	
Age............	7 days	7 months (fetal)	9 days	Birth	Birth	30 days (fetal)	4 days	
Per cent of dry substance....	20.21	19.25	20.33	19.40	21.46	20.60	20.2	
Age............	15 days	New born	14 days	28 days		14 days		
Per cent of dry substance....	27.14	27.35	26.19	27.60		27.70		
Age............	21 days	56 days		28 days		21 days	Grown	
Per cent of dry substance....	29.39	29.85		29.30		29.2	29.99	
Age............	Grown (294)	Grown	83 days	100 days		Grown		Grown
Per cent of dry substance....	34.7	32.4	33.35	31.10		30.77		32.92

Indeed the percentage of solids in the rat at birth corresponds with that of the foetal life of the other mammals here recorded. This immaturity of the body at birth and subsequent rapid growth of the body during the first three weeks must naturally call for an appropriate quality and quantity of the milk itself.

The present results on the growth of dry substance agree with those obtained by Lowrey ('13) who has not only determined the dry substance of the entire body of the albino rat, but also that of its various parts and systems. The only disagreement between the results of Lowrey and of myself is exhibited by the percentage of dry substance of the body at birth. Lowrey gives 11.7 per cent of solids, while I find 12.8 per cent. I am unable to explain this discrepancy at the present moment.

4. The composition of the dry substance

Definitions of the terms used for the fractions of the dry substance recorded in table 6 are given in the following paragraphs.

TABLE 6

Showing the percentage composition of the solids of the albino rat during the life span, as represented by the residue, fats, organic extractives, soluble salts and fixed salts

	AGE IN DAYS							
	Birth	7	15	22	28	35	42	294
Residue	56.9	42.0	39.9	38.8	38.6	44.9	44.4	44.5
Percentage of moist weight	7.3	8.5	10.8	11.4	11.7	13.2	13.6	15.5
Fat	14.2	35.4	39.2	36.6	37.7	25.9	27.1	16.5
Percentage of moist weight.	1.8	7.2	10.6	10.8	11.5	7.6	8.3	5.7
Organic extractives	16.4	12.8	12.8	14.8	13.8	18.6	16.9	28.2
Percentage of moist weight	2.1	2.6	3.5	4.3	4.2	5.5	5.2	9.8
Soluble salts	6.6	4.6	3.0	3.2	3.3	1.5	2.7	2.5
Percentage of moist weight	0.8	0.9	0.8	0.9	1.0	0.4	0.8	0.8
Fixed salts	5.9	5.2	5.2	6.7	6.5	9.2	8.9	8.3
Percentage of moist weight	0.7	1.0	1.4	2.0	2.0	2.7	2.7	2.9

1. Residue. The residue is represented by the solids from which all substances soluble in boiling alcohol and in water, as well as the salts, have been removed. Thus the residue as here defined represents practically all the protein substances. As will be seen from the table, the residue expressed as a percentage of the moist (= total) weight increases steadily from birth onwards. However the residue considered as a fraction of the dry solids is highest at birth and diminishes gradually until the end of the lactation period, after which it rises again. This change in the relative residue content is probably caused by the variations in the proportion of fat.

2. Fat. Fat is represented by the substances, soluble in boiling alcohol, from which the water soluble organic extractives and salts have been removed. From the table we find that the rat at birth has a very small amount of fat. The fat however increases very rapidly during the first week and then at a somewhat slower rate. This change in the fat content is seen whether the fat is considered as a fraction of the moist weight or of the dry solids. This very rapid increase of the fat during the lactation period seems to be accounted for by the very high content of the fat in the milk. The diminution of the fat, after the lactation period is over, is very noticeable.

This may be partly due to the rapid formation of the supporting system or tissues in general or, more probably, to an increased metabolic activity. It must be stated that so far as our experience goes, the rats are seldom fat as the age ot five or six weeks.

3. Organic extractives. All water soluble substances from which the salts have been removed are here called the organic extractives. Since the organic extractives are mainly intermediate metabolic products of the various organs and tissues, we should expect their variation to be more or less in conformity with those of the residue. This expectation is well realized since they increase steadily as the body weight increases (see per cent in terms of moist weight). When this group is considered as a percentage of the dry substance, it is found to be highest at birth and then to decrease towards the end of the lactation period, after which there is another steady increase. In general therefore its variation is related to the variations of the residue, and is an index of growth and activity.

4. Soluble salts. The salts so designated were obtained from all the extractives made both with water and with alcohol. The soluble salts exist in the body probably either dissolved in the various body fluids or loosely combined with various tissues. The salts, considered as a percentage of the moist weight, are nearly constant and the value is also the same as that for the milk (stomach contents). I am unable to explain this curious phenomenon. The soluble salts as a fraction of the dry substance decrease slowly but steadily from birth onwards.

5. Fixed salts. The solids from which fat, organic extractives and soluble salts had been removed were incinerated and the ash thus obtained is here called the fixed salts. These salts therefore represent practically all salts present in the osseous system. Thus in as much as the fixed salts represent practically all bone ash, we should anticipate an increase of this fraction from birth onwards. This appears to be the case.

5. The composition of the entire body of various mammals at different ages

It should be recalled that the protein substance given by most other investigators is equivalent to the weight of 'nitrogen × 6.25.' Since the organic extractives in the present investigation are mainly nitrogenous substances, the residue in the present case and the protein substance as determined by other methods should not theoretically agree, the latter being somewhat high. As to the fat, most investigators designate alcohol and ether extracts collectively as fat, while the fat in the present case in represented by the alcohol-ether extractives minus organic extractives and salts. Thus the two data should not theoretically agree. To meet the difficulties just mentioned, and also to make as nearly similar as possible the two sets of data, the organic extractives were added to the residue and to the fat in the proportions of two to the residue and one to the fat. This correction is however open to further modifications. Fortunately however only comparatively small quantities of the organic extractives and salts are extracted by the alcohol and ether and thus the two kinds of data, our own and those from the literature, may be compared directly for the purpose of a rough determination. I have however adopted these corrected values where the present results on the rats were compared with the data given by the other investigators on various animals (table 7).

I now wish to compare the analysis of the rats with the analyses of several mammals as recorded by other investigators.

With the exception of those for the rat, the data which are given in this table have been obtained from papers published at various times by several different investigators (Aron '15). It must be remembered therefore that the data are highly hetero-

TABLE 7

Showing the data on the growth of protein, fat and ash in various mammals, associated with the growth in body weight, as well as the changes in the water content of the body

SPECIES	AGE	BODY WEIGHT	WATER	SOLIDS	PROTEIN	FAT	ASH
		grams					
Man	5½ months fetus	508.0	86.9	13.1	8.1	1.3	2.4
Rabbit	21 days fetus	11.7	86.3	13.7	8.5	2.1	1.4
Rat	Birth	4.3	87.2	12.8	8.7	2.5	1.6
			86.8	13.2	8.4	2.0	1.8
Man	7 months premature birth	116.9	81.8	18.2	10.4	3.2	2.9
Rabbit	Birth	38.4	79.4	20.6	11.5	4.9	2.5
Dog	2 days	267.0	80.5	19.5	13.2	1.9	2.3
Cat	4 days	152.9	80.0	20.0	13.6	2.7	2.4
Pig	Birth	1,726.0	80.0	20.0	13.4	1.4	1.8
Rat	7 days	10.2	79.8	20.2	10.1	8.0	2.0
			80.3	19.7	12.1	3.7	2.3
Man	New born	3,058.0	72.7	27.3	11.8	10.7	2.6
Dog	44 days	1,460.0	72.8	27.2	14.5	10.3	2.7
Cat	14 days	515.0	73.8	26.2	15.0	7.1	2.5
Rat	15 days	13.5	72.9	27.1	13.1	11.8	2.2
			73.1	26.9	13.6	10.0	2.5
Man	28 days	3,838.0	69.7	30.3	13.9	13.1	3.1
Rabbit	Grown	1,480.0	69.2	30.0	18.2	7.8	5.8
Dog	52 days	2,011.0	70.1	29.9	13.6	12.1	2.9
Rat	32 days	47.6	70.1	29.9	15.7	11.1	3.2
			69.8	30.2	15.4	11.0	3.8
Dog	49 days	1,985.0	64.6	35.3	13.4	18.9	2.8
Cat	83 days	1,389.0	66.7	33.3	20.1	7.9	3.3
Guinea-pig	Grown	424.0	67.1	32.9	19.9	10.0	4.4
Rat	294 days	277.5	65.3	34.7	22.1	9.0	3.7
			66.2	33.8	18.9	11.5	3.6

geneous as to the method of analysis, as well as to the condition and number of animals, and all of these facts should be considered when interpreting the data.

The data are arranged in five groups. In group 1 the animals whose bodies give the percentage of water which is nearly the same as that given by the albino rat at birth are contrasted with one another without regard to either calendar age or body weight. Similarly in the four remaining groups the animals whose bodies give an amount of water nearly the same as that of the rat at 7, 15, 32 and 294 days respectively are also compared. The table may be analysed from various standpoints.

1. *Age and body weight.* Despite the fact that the percentage of water in the body is practically the same in the several entries composing a group, the corresponding ages or body weights vary widely in the different species.

This fact may be taken to mean that the different species have a dissimilar rate of growth in body weight and require different intervals of time in order to pass through the several values for the percentage of water which lie between the two limiting values of 88 per cent and 65 per cent found in all mammals so far examined. Thus the percentage of water on the one hand and body weight or age on the other are not similarly related in the mammalian series.

2. *Percentage composition of solids.* On the other hand if we compare the percentage composition of the solids (protein, fat and ash) given by the various mammals, when they possess a like percentage of water, a surprising uniformity is shown. In general, similarity in the percentage of water gives similarity in all the other main chemical components. A slight tendency to an increased variability as shown by the older animals seems due to several analytical difficulties—represented by complete drying and the extraction of fat. Thus it appears probable that a uniform technique and also a vigorous selection of healthy individuals would tend to smooth out the irregularities now shown in this table.

Despite the presence of some irregularities, we see plainly that following the progressive diminution of the percentage of water, the percentage values of protein, fat and ash increase regularly. Furthermore, this reciprocal relation of water and other chemical groups is so harmonious that we are strongly tempted to conclude that there is a definite quantitative relation similar in all mammals in their chemical make up at equivalent ages.

We infer from the above that the bodies of all the mammals, whether carnivorous or herbivorous, during their growth pass through identical phases of chemical alterations, though the time required to complete the life cycle may be widely different in different species. The corresponding phases of chemical alteration appear to follow the water content of the body. Consequently we may conclude that the percentage of water is an indicator of corresponding phases of chemical alteration of the mammalian bodies, while neither the calendar age nor the body weight of the animals can be used for the same purpose.

It has been noted by Donaldson ('06) that the normal span of human life is about thirty times that of the albino rat. This conclusion was based on the observation that man requires thirty times as many days to double his birth-weight as does the rat (p. 26, table 3) and on the assumption that the average maximum age of man is 90 years, while that of the albino rat is three years. Donaldson noted further ('08) that the graph of growth for the brain in weight is similar both in man and in the rat if the age intervals of man are reduced one-thirtieth, and further the brain weight of adult man (1400 grams) is reduced to the adult brain weight of the rat (2 grams). This result shows then that the two main phases of growth (postnatal cell division and the swelling process) in these two forms are similar at corresponding ages.

In his third communication Donaldson ('10) shows not only that these growth phases agree in man and in the rat, but also the percentage of water in the brain agrees in the two species at equivalent ages. From this we infer that the percentage of water in the brain may be taken as the mesaure of the equivalent ages of man and of the rat.

Very recently Mayer and Schaeffer ('14) studied the relation between the amount of water and of various lipoids in several visceral organs in various mammals and reached the conclusion that a certain definite parallelism exists between the degree of imbibition of water and the ratio "cholesterine—fatty acids," or "cholesterine—lipoid phosphorus."

Mayer and Schaeffer call these relations "The lipocytic coefficient of the tissue" and found the coefficient identical in all animals examined when the imbibition of water was identical. The present investigation, as has been already stated, demonstrates that the percentage of water is the index of equivalent phases of chemical alteration of the entire body. Thus my own investigation not only supports the views presented by Donaldson, but also indirectly the views held by Mayer and Schaeffer, but at the same time extends the significance of water content in the living organism as represented by the mammals during the life span.

CONCLUSIONS

The entire body of the albino rat was analysed at birth and at 7, 15, 22, 28, 35, 42 and 294 days and the variations according to age were determined for water and solids, and from the solids for the following chemical components: protein, fat, organic extractives and salts. The stomach contents o' the young rats fed by the mother's milk exclusively were analysed also. Analysis of the stomach contents suggests that the milk of the albino rat is highly concentrated and rich in fat.

1. The percentage growth of the solids reaches nearly a maximum (30 per cent) while the young are still nourished almost entirely by the mother's milk (end of the third week). At the end of 42 weeks the additional growth in solids is only slightly over five per cent.

2. The components of the solids show the following age variations:

Protein. The protein content is highest at birth and diminishes gradually until the end of the lactation period, after which it rises again slightly.

Fat. The fat content rises rapidly during the lactation period, after which it diminishes steadily.

Organic extractives. The organic extractives decrease rapidly towards the end of the lactation period, after which there is another steady increase. In general the variation in the organic extractives is similar to the variation in the protein.

Salts. The salts show a slight progressive reduction during the lactation period, after which they increase steadily.

3. When the chemical composition of several mammals are compared with one another it appears that the bodies of these mammals during growth pass through similar phases of chemical alteration, so far as the water, protein, fat and ash contents are concerned, and that the percentage of water is an indicator of the corresponding phases of the chemical alteration in different species, while neither the calendar age nor body weight of the animals can be used for the same purpose.

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EXPERIMENTAL STUDIES ON THE ORIGIN OF VASCULAR ENDOTHELIUM AND OF ERYTHROCYTES

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EIGHTY-NINE FIGURES

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INTRODUCTION

The following contribution represents a résumé of three years of experimental study in which an attempt has been made to analyze certain of the processes involved in the genesis of some of the vascular tissues. For constant encouragement and most helpful criticism I wish to acknowledge my deep indebtedness to Prof. Charles F. W. McClure, under whose guidance the work was undertaken. I am especially grateful for the generosity with which he has furnished me with necessary funds and apparatus for the undertaking of the problem at hand. I have also been so fortunate as to have recourse to the valuable counsel of Prof. E. G. Conklin, whose advice has been of great help to me.

PART I. THE ORIGIN OF ENDOTHELIUM

In many respects the development of the vascular tissue is unique. Its investigation is attended with certain peculiar difficulties which have hindered the solution even of some of the least involved problems in this field of development. Within recent years the origin of the endothelium which lines the haemal and lymphatic vessels has perhaps been the central problem of anatomical investigation. Over this question there has been an extended controversy; even now it would be premature to say that all the problems confronting us are completely settled.

The principal points of difficulty with which one must reckon in consideration of this peculiarity of endothelial tissue are raised by these questions: Does physical continuity of endothelial cells necessarily imply genetic continuity? Conversely, is it possible that cells out of continuity with already formed endothelium can ever become continuous with and a part of that endothelium? It has been observed that in most classes of vertebrates the site of endothelial formation is the yolk-sac. Later, endothelium is found progressively nearer the embryonic body and finally inside the embryonic body. As has been so many times stated, there are two views concerning the nature of this endothelium which is found associated with the intraembryonic tissues. One view is that the earliest vascular tissue grows toward the embryo, and on reaching the embryo's body permeates the intraembryonic tissues in a centrifugal manner, forming the entire lining of the haemal and lymphatic systems. This view has been designated as the angioblast theory of Wilhelm His. Let us consider a few quotations from certain of the supporters of this theory; in this manner we can study firsthand the conceptions of these observers concerning the implications of this so-called ingrowth or extension theory.

I. THE ANGIOBLAST THEORY

Minot's conception of the angioblast (Keibel and Mall, Human Embryology, vol. II, pp. 498–9) is as follows:

> Comparative embryology teaches us that the first vessels appear on the yolk-sac collectively and at one time. They form a unit anlage, which we call briefly the angioblast according to the suggestion of His. . . . . I am inclined to think that the angioblast . . . . forms itself not through the transformation of mesodermic cells already present, but from the layer of yolk-cells, and from a reticulate grouping of themselves between the middle and lower germ-layers. The angioblast probably maintains its complete independence throughout

life. In other words, it is probable that the endothelium of the blood-vessels (and of the lymph-vessels) and the blood cells at every age are direct descendants of the primitive angioblast.

The following is from Evans (Keibel and Mall, vol. II, p. 571):

> In embryos of the higher vertebrates the first cells which can be identified as standing in any relation to the vascular system appear in the form of localized thickenings of the extra-embryonic mesoderm lying next the entoderm of the yolk-sac. These constitute the so-called vascular anlagen and typically undergo a gradual differentiation into two or more definite cell-types, blood cells on the one hand, and endothelial cells on the other. . . . . It is important, then, to distinguish vessels which have arisen through sprouting of other vessels in contrast to vessels whose endothelium has been contributed directly from the neighboring mesoderm. Even on the yolk-sac these latter vessels are not numerous, for they occur only at the site of the so-called anlagen, and the main mass of the vitelline capillary plexus arises from the extension and frequent anastomosis of these primary vessels. . . . . In birds it has been possible to establish beyond all doubt that most of the aorta descendens is formed from the medial margin of the vitelline capillary plexus.

Bremer (3) agrees with the angioblastic view. He believes, however, that the injection method is inadequate for mapping out the extent of the vascular tissue, even in case of the caudal aorta, as the establishment of its lumen is not a continuous process, so that isolated cavities actually exist, but the cells of their walls are in genetic continuity. He states (p. 114) that "it was the observance of these isolated spaces which later fuse to form large vessels, that lead to the often repeated statements of Rückert and Mollier and others that the dorsal aortae arise in situ from cells of the mesoderm."

In 1914 (4) his conception of the angioblast is much modified (p. 462):

> I think it well at this point to define more accurately what I mean by angioblast cords, especially since I believe that their recognition may help to explain the frequently described endothelial spaces unconnected with any injectible vessel. The angioblast cords are apparently solid cords of cells connected end to end or in small groups, running between the processes of the surrounding mesenchyme cells, when these are present, often touching them, without actually fusing with them. The diameter of the cords is never as small as that of the

mesenchymal process, though it is often less than that of the cord nuclei. The cords tend to form nets by anastomosis of larger mesh than the mesenchymal net, and angiocysts wherever space is given. They are usually sharply defined from the surrounding tissue, and may show an extraintimal space.

In this sense the angioblast is not necessarily the unit vascular anlage which grows into the embryo from the yolk-sac. Its only prerequisite is that it shall grow continuously (from one or many sources) through the embryonic tissue as 'solid cords of cells.'

It is to be hoped that the term 'angioblast' may never be applied to any structure except to this theoretically early differentiated, precociously segregated, yolk-sac, unit vascular anlage which has been claimed to give rise to all the vascular tissues. If no such exclusive and sole 'unit anlage' exists, then the term needs and deserves to be discarded. It has been used in the literature for such a great number of years that its meaning is very definite and well established. The term is so intimately related to the concepts of specificity and ingrowth from the yolk-sac of all vascular tissues that it can never, with propriety, be attached to any embryonic structure except the early yolk-sac vessels. It is to be hoped that no observer will ever find it necessary to designate individual mesenchyme cells as 'angioblasts,' even provided he should become convinced that such cells are vasofactive. The term 'angioblast' implies a 'unit anlage;' it implies a collectively multicellular tissue, and therefore can not properly be used in connection with a single cell. If there be such a thing as angioblast, there is but one angioblast and it has but one method, place, and time of origin. To no isolated cell should be attached a term which for decades has been applied to a group of yolk-sac blood-vessels. Strictly speaking it would be inconsistent to designate as 'angioblastic cords' any solid forerunners of intraembryonic vascular tubes, even though the ingrowth theory were correct. Angioblast can not properly be applied to solid or tubular endothelium which has arisen by sprouting; the term implies the original anlage which does the sprouting, and which does not, according to the angioblast theory, arise from preexisting vascular tissue; it does not include the sprouts, or the sprouts of sprouts.

Finally the view of Rabl might be mentioned. He is responsible for the dictum "Endothel stammt nur von Endothel." Taken literally such a statement would relegate vascular endothelium to a category in which the germ cells, and perhaps some of the most primitive organisms are generally regarded as unique because of their supposed immortality.

II. THE LOCAL ORIGIN THEORY

The view opposed to the angioblast theory is that of local origin. According to this view, mesenchyme may, in practically any region of the body, transform into vascular tissue. The cells which bound an intraembryonic blood-vessel are not in direct lineage with those which line the early vessels on the yolk; they have not come into being as ingrowths from the early yolk-sac vessels or 'angioblast;' they have not necessarily come from preëxisting endothelial cells, though some of them may have had such an origin, inasmuch as local origin does not preclude the possibility of growth during or following the process of local vascular formation. Additions to endothelium already formed may take place by (1) proliferation of cells already formed, (2) addition of single mesenchyme cells, (3) addition of solid cell aggregates, (4) addition of already formed endothelial cavities the lining cells of which have differentiated locally, in and from the mesenchyme, and (5) by the active migration and alignment of single mesenchyme cells to form vascular cavities. The local origin theory holds that blood-cells are not necessarily descended from a primitive yolk-sac 'angioblast,' but that mesenchyme within the embryonic body is capable of giving rise to blood cells. Advocates of the local origin theory do not believe that the vascular anlagen are necessarily differentiated at a very early stage of development, as claimed by His, or collectively and at one time as stated by Minot; advocates of the mesenchymal theory recognize that there are certain regions in which a precocious production of vascular tissues takes place, but they claim that such regions are not the only regions in which such tissues are formed. Advocates of the local origin theory recognize various intraembryonic regions in which there is a first-hand production of vascular tissues, even relatively late in ontogeny, and quite independent of such processes in the yolk-sac. The angioblast theory regards endothelium as a tissue of high specialization, quite foreign in nature to mesenchyme and quite removed from it genetically. The local origin theory claims that mesenchyme can transform into endothelium, and that endothelium can transform into mesenchyme.

The theory of local mesenchymatous origin of endothelium dates back to the work of Goette (15) and Reichert (56). Within more recent times, Rückert and Mollier (60) and their students have chiefly constituted the European School who maintain that endothelium develops in situ. Maximow (31), Bonnet (2), von Felix (13) and others have also supported this view. In this country, Huntington (19, 20) and McClure (32-37) and their students have stood practically alone as sponsors of the local origin theory.

It is of interest to note some of the methods which have been employed by the advocates of these conflicting views. Investigations supporting the angioblast theory have sometimes been based on studies of serial sections, frequently on relatively late growth stages in the living tissues of amphibian larvae and other tissues, and especially within recent times, on injections. Certain objections to the results obtained by these methods have been raised by the opponents of the angioblast school. Especially Huntington, McClure, and Schulte have urged that intravital study of late capillary plexuses, as has so often been made, [Platner (47), Remak (57), Kölliker (25), Langer (26), Clark (7), etc.] is merely a study of endothelial proliferation and not a study of its genesis. To quote McClure (36), pp. 61–2):

> We all recognize the fact that endothelium, like other tissues of the body, is capable of growth after it has once been formed. In no other manner could we account for the increase in size which blood-vessels undergo in the embryo. . . . . It is also possible for anastomoses to be formed between different vessels by means of growth or sprouting of their endothelial walls so that, in some cases, an increase in their extent through growth may actually take place. . . . . From whatever standpoint it may be considered, the growth of endothelium is of secondary significance as regards the problem at hand, since the main question at issue does not concern the possibility that endothelium may or may not grow, but rather how the endothelium is formed that does the growing.

The injection method has also been criticized as a method capable of giving only one result—a negative result with reference to the possibility that non-continuous vascular lumina exist. Huntington and McClure have repeatedly called attention to the fact that injection will do nothing more than demonstrate continuous cavities. Those who believe the injection method to be adequate, maintain that any cavity in the uninjectible periphery is necessarily a non-vascular cavity or an artifact. In case of the early vessels in the chick, the injection method should be able to demonstrate all endothelium from the moment that the independently formed portions of the angioblast or 'unit anlage' have become confluent. In this connection Schulte (63, p. 25) states:

> From this moment . . . . there is a sudden and abrupt end to the production of discrete anlages, contrary to what one would expect from the general transitions in natural processes.
>
> But in view of the many anlages admittedly present in the splanchnopleure and in the embryo, and of transitions almost universally observed in natural processes, the possibility must be conceded that a belated vesicle or two of endothelium might conceivably escape a sudden annexation to the injectible system. It becomes of interest to determine its status after injection has been practised and it has failed hypothetically to be injected. A moment before, had it been observed in vitro, or had it been sectioned, it would have appeared like any other of the discrete anlages in the 'anlage.' But the test of injection made, it at once ceases to have a future, it has become an artifact, or if pertinaciously insisted upon, a Mayer-Lewis anlage. Because . . . . it failed of concrescence prior to the moment of injection it is absolutely devoid of vascular potentialities and could never, had the injection been omitted, have joined its fellows and participated in the formation of the vascular system. . . . . (p. 7). We may recognize, or refuse to recognize the uninjectible periphery. If we recognize it, the method of injection becomes not only partial and incomplete, but subordinate to the methods which reveal all its findings and in addition, enlarge our field of observation.
>
> . . . . (p. 27). To . . . . insist that the limited field explored by injection is alone accessible to investigation is not to assert the primacy of the injection method, but to fall back upon an ancient logical device known as *petitio principii*. . . . . (p. 26). There is, however, no reason to assume that injection has reached the acme of delicacy, great as is the skill which has been developed in its use. It is conceivable that some day it will be possible to inject a portion of the 'anlage' before its admittedly discrete vessels have fused—even a small fragment might be injected alone, the dorsal aorta in the head, say, or the umbilical vein. This would then become to the injectionist the only source of the vascular system. The uninjected parts of the

anlage would be demonstratedly not vascular anlages at all, but artifacts or 'Mayer-Lewis anlages,' and no study by slide or section could establish their right to serious consideration.

It is of interest in this connection to note that McClure (33, 34, 36) has been able actually to inject a discrete lymphatic anlage—the sub-ocular lymph sac in the trout—independently of the main systemic lymphatics, veins, and arteries, yet it is inconceivable that these latter vessels, as revealed in section, could be regarded as non-vascular spaces.

In defense of the injection method, E. R. Clark (8) has attempted to show by tests the adequacy of injection as compared with serial sections, which are often relied on by those who believe in the local origin of endothelium. He injected small lymphatics, drew their outlines, then sectioned the tissue and reconstructed the vessels. He found that the lines formerly occupied by the continuous vessels were occupied by series of isolated, black, bead-like areas. Doubtless one does well to admit that shrinkage actually takes place in preserved tissues, but doubtless also there is no other case in the literature where the series of isolated spaces comparable to these are individually figured in solid black. If one should combine complete injection with serial sectioning, and find lines of discontinuous injected spaces he would probably not commit the error of regarding them as always having been out of continuity. In all probability the only recorded case of an injected isolated vascular space, besides those figured by Clark, is that of McClure's subocular sac; this was observed in the living condition to be independent, and was injected directly.

As already stated, those who believe endothelium to form entirely by proliferation of a small, restricted yolk-sac anlage have been strengthened in their belief by observation of the sprouting of living endothelium. On the other hand, direct observation of the mesenchymal synthesis of endothelium is by no means wanting. As early as 1885 Wenckebach (77) described with great accuracy the remarkable 'Bewusstsein' with which single mesenchyme cells migrate by amoeboid movement to arrange themselves into endothelial tubes on the teleost yolk-sac; he also observed the same process in the embryonic body, but here the process was a re-arrangement rather than an extensive migration. So thorough were the observations of Wenckebach that later observations on the living telost yolk-sac by Raffeale (50), Stockard (66), and myself (52, 53) have added little of importance to our knowledge of the process given us by Wenckebach.

III. THE EXPERIMENTAL WORK

> The great problem of development is to determine as far as possible, the sequence and origin of differentiations and the extrinsic and intrinsic forces by which they may be modified. Much has already been accomplished in tracing the sequence of morphological differentiations to earlier and earlier stages of development, but relatively little has been learned as to the nature and causes of differentiation itself. By the observation of purely normal processes one sooner or later comes to a place beyond which he can make little if any progress in the study of such problems; but the history of biology in the last twenty-five years has shown that much may be learned by the comparison of normal and abnormal processes, and especially by the combination of observations of normal processes with experiments which may be varied indefinitely.—Conklin.[1]

Prior to the year 1913 it might well have been said that the status of the controversy over the origin of endothelium was that in which the observation of the normal process had practically reached its limits, so far as the general acceptance of one view or the other was concerned. Those who believed serial sections to be adequate were not convinced by those who believed in the adequacy of injection, and vice versa. Also, those who had seen the sprouting of living endothelium failed to take into account the evidence at hand that isolated mesenchyme cells had been seen to form endothelium. The question resolved itself into one of methods; that certain definite results could be obtained by each of these diverse methods was no longer questioned by any one. As affirmed by Bartels concerning one of the methods, we might well affirm concerning the questions involved in all the methods employed; they were "philosophical, and not anatomical questions." The account of the development of the vascular system as given in Keibel and Mall's Human Embryology, 1912, probably represents the consensus of opinion among the majority of American and European anatomists up to 1913—namely that the vascular tissues were of angioblastic origin. A thorough survey of the literature and most recent text books does not lead one to agree with Stockard (66, p. 587) that the recent defenses (Stockard considers them 'revivals') of the ingrowth theory were prompted by purely 'literary reasons.' Also it is improbable that we have reached the era in which philosophical questions can be divorced from the interpretations of the results reached by our methods.

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[1] Journal of the Academy of Natural Sciences of Philadelphia, vol. 15, Second Series, 1912, pp. 503–4.

It may well be said that the thirtieth session of the American Association of Anatomists, 1913, marks a new epoch in the endothelium controversy. Schulte at that time showed that the anlagen of the umbilical vein develop locally from parietal mesoderm entirely away from splanchnopleuric vascular anlagen. McClure showed that an isolated lymphatic anlage could be injected directly and independently. But in addition to these significant morphological contributions there stands out in importance the experimental work of Miller and McWhorter (40) who had obtained blood-vessels on the operated sides of chick embryos, one side of which had been excised from the yolk-sac blastoderm. This work is not the first experimental investigation of the problem, but it marks a revival of that sort of investigation of endothelium; it marks the beginning of a serious appreciation of such work, and a concession on the part of many anatomists that the local origin theory is not a priori untenable.

As already stated, the work of Miller and McWhorter was not the first attempt to utilize the experimental method in the solution of problems having to do with the origin of the vascular tissues. In the year 1887 there were three experimental investigations bearing on such problems—the investigations of Budge, Gerlach, and Uskow.

Budge (5) proposed to confirm the view of His by destroying the germinal disc of unincubated eggs, leaving the germ-walls intact so that the latter might demonstrate its independence as the source of the vascular tissue. Gerlach (14) tried to determine experimentally whether primitive-streak mesoderm or parablast was the source of the vascular tissue; this he tried by preventing the development of the primitive streak. He was unable to obtain decisive results. Uskow (72) likewise attempted to arrest the development of the mesoderm, but was unsuccessful.

The work of Gräper and Hahn along these same lines furnishes a foundation for the subsequent experimental work on vasculogenesis. Their observations have, unfortunately, received little attention from later investigators; for this reason it seems advisable to consider rather minutely their methods and results.

Gräper's work

Gräper's investigation (16) is primarily an investigation of the process of heart-formation, in which the main purpose is to show that the heart, in accordance with the 'Rablsche Erklärung,' is essentially a fused pair of vitelline veins, and its efferent vessels are formed in genetic continuity with its own endothelium. The work attempts to show the explicability of double and multiple hearts on the basis of a failure of the mesial fusion of the endothelial tubes, which originally were laterally located. Evidently Gräper regarded our knowledge of heart formation as somewhat uncertain and theoretical. A second purpose of Gräper's work, which was not essentially experimental, was to prove that the earliest vascular tissues on the yolk-sac are of entodermal origin; that they are formed from entodermal cells which separate from that layer, invade the germ-wall, and emerge to form endothelial cells and blood-cells; that at least some of the invading cells become 'Dotterträger' which participate in the formation of vascular tissues and in their nourishment. The work attempts to disprove the view of Rückert and Mollier that the vascular tissue is derived from primitive-streak mesoderm which is proliferated into or migrates into the vascular area and possibly into the germ-wall.

Ordinarily when blood-islands are first recognizable, they are found lying between the mesoderm and the germ-wall. As Rückert has observed, their position when first recognizable gives little clue to their ultimate origin. Gräper points out that Rückert's study of avian blood-islands was confined largely to the posterior portion of the blastoderm, where he (Gräper) believes the picture is not clearly marked; his own studies deal mostly with the anterior portion of chick blastoderms in which the head-process has a length of about one millimeter. In this location he was able to find isolated blood-islands in a region not yet invaded by laminated mesoderm. He sometimes found blood-islands more than a millimeter distant from any mesoderm. Such blood-islands lay between germ-wall entoderm and ectoderm. Relying upon the extreme improbability that their origin should ever be regarded as ectodermal, he believes that such blood-islands furnish conclusive evidence for the entodermal origin of the vascular tissues.

Rückert (Hertwig's Handbuch, Bd. 1, S. 1180) writes:

> Worin besteht aber die Bedeutung des Dotters für die Blutbildung der Wirbeltiere? Auch hier gibt das Ei des *Platydactylus* einen Fingerzeig. Da die Verdickung des unteren Blattes erst nach dem Erscheinen der Blutanlagen auftritt bezw. (vorn) zu voller Ausbildung gelangt, so kann dieselbe nicht die Aufgabe haben, den Entoblast zur Abgabe von Zellenmaterial an die Blutinsel vorzubereiten. Das Wesen des Vorganges kann also nicht in einer Zellen sondern nur in einer Stoffabgabe des Dotters an die Blutanlagen beruhen.

Gräper dismisses this observation of Rückert's with a statement that it apparently rests "auf Grund vergleichender Forschungen." If it be true, as in the instance cited by Rückert, that blood-islands may develop in a region where there is as yet no thickened entoderm, the isolated blood-islands of Gräper are not all-significant, albeit they display a condition of extreme interest. If blood-islands can develop at a distance from mesoderm, and at a distance from thickened entoderm in another, it is evident that no valid generalization can be made concerning either of these layers as the sole source of the vascular tissue.

Jan Tur (71) described vascular anlagen completely isolated from entoderm, mesoderm, and from ectoderm. Yet he stated: "Les éléments de ce 'parablaste accesoire' qu'on pourrait désigner sous le nom de 'parablaste sous-germinale' peuvent donner naissance à de vraies formations vasculaires dont la valeur est nulle, mais qui prouvent la théorie de l'origine entodermo-parablastique des vaisseaux sanguins." (Parablaste here refers to germ-wall.) If this case reported by Jan Tur be of significance, one might, in following Gräper's line of reasoning, be compelled to enunciate a new germ-layer. Perhaps a most feasible explanation of these isolated blood-islands is the assumption that we are here dealing with migrating mesodermal (mesenchymal) cells; such cells may perhaps arise either from mesoderm, entoderm, or both. Once such a cell has arisen from entoderm or mesoderm, it might well be designated as mesoderm or mesenchyme. As will be considered later, the work of Wenckebach, Raffeale, Stockard, and the writer has shown, the teleost yolk-sac, entirely devoid of true entoderm, furnishes an excellent place in which to study the actual migration of living mesenchyme cells and their transformation into vascular tissue. A study of such migrating cells might convince one that the traversing of a distance of 1 mm. by mesenchyme cells would not be out of the question in case of Gräper's isolated blood-islands.

Rückert points out the possibility that even though the cells emerging from the germ-wall may be closely approximated to the entoderm, they may conceivably have come from mesoderm. Gräper answers this by maintaining that ultimately all the mesoderm in question came from entoderm (which is certainly not true in case of primitive-streak mesoderm in the chick); Gräper also maintains (p. 381) that even if the cells in question had come from mesoderm, they should necessarily be regarded as entoderm when they were in association with that layer, "so könnte man doch unmöglich von mesodermaler Bildungsweise reden." Such confusion at least shows the need of a definite terminology.

van der Stricht (67) described yolk-laden cells in the mesoderm, and believed that such cells had taken on a yolk-content by means of protoplasmic processes which reached down into the yolk. This offers a possible explanation of the "Dotterträger" of many authors. In some cases such protoplasmic processes might not be preserved in fixed material.

On one point most observers are agreed. Whatever may be the origin of the prevascular cells of the yolk-sac, they leave, perhaps by migration, one or some of the laminated primitive layers before they acquire their vascular characteristics. Such active movement is more characteristic of mesodermal than of entodermal cells.

Prior to its publication, the work of Hahn was known to Gräper. As will be considered later, Hahn attempted to prove that primitive-streak mesoderm is the source of the yolk-sac blood-islands. This he did by destroying the region lateral to the posterior portion of the primitive streak. He obtained embryos whose blastoderms on that side were in most cases entirely devoid of blood-islands. Gräper performed these same experiments, but claimed that it is necessary to destroy not only the existing vascular tissue at the operative stage, but also the germ-wall as well; he claims from this that the germ-wall and not the primitive-streak mesoderm is responsible for the vascular tissue.

The work of Gräper also deals with the origin of intraembryonic vessels. On the operated sides of his experimental chicks he obtained vitelline veins, endocardia and dorsal aortae. In the description of his embryo 'G' he gives the line of reasoning by which he interpreted his results as not supporting the views of Rückert. On p. 396, describing the operated side, he states:

> An der Stelle nun, wo man die zweite Herzhälfte bezw. die Vena omphalomesenterica erwartet hätte, sieht man zwischen Entoderm und Mesoderm einen rundlichen Zellhaufen (Z), der ganz den Eindruck einer Blutinsel macht. Verfolgt man diese Masse nach vorn und hinten, so erhält man von Zeit zu Zeit Bilder, die genau mit denen übereinstimmen, die Blutinseln geben, die in der Bildung bluthaltiger Gefässe begriffen sind. Vor der vorderen Darmpforte kann man eine Verbindung mit dem 'Herzen' der anderen Seite nachweisen. Es macht also den Eindruck, als ob hier eine autochthone Bildung eines Gefässes stattgehabt hätte. Dies ist auch tatsächlich der Fall, nur nicht im Sinne derer, die behaupten, dass alle grösseren Gefässe in loco entständen. Es kommt hier vielmehr eine Zufälligkeit in Betracht, die nachzuweisen mir in diesem Falle glücklicherweise gelang. Verfolgt man nämlich die Serie durch eine grössere Anzahl von Schnitten nach hinten, so sieht man diesen Blutinselstrang in ein kleines Divertikel übergehen, das an der Stelle liegt, wo Entoderm und Ectoderm vernarbt

> sind. Dieses Divertikel, das Fig. 24 darstellt, enthält noch reichlich in Resorption begriffenden Dotter. Hieraus sehen wir, dass es bei der Operation nicht gelungen war, die Area opaca vollständig zu entfernen, es waren vielmehr noch Keimwallreste central von der Narbe geblieben. Hierzu kommt eine starke Verschmälerung der Area pellucida, wie sie ja stets an den operierten Embryonen beobachtet wurde. Durch diese Umstände war es möglich, dass die Keimwallreste in den Embryo einbezogen wurden. . . . . Es bestärkt dieser Embryo also keinwegs die Theorie der lokalen Entstehung der Gefässe in Embryo, sondern ist vielmehr, besonders mit dem Embryo C zusammen, ein Beweis für die Entstehung der Blutinseln aus den Dotterzellen.

In my own experience I have found too that if embryonic tissue be left in communication with an area vasculosa bearing blood-islands the vascular tissues in the two regions invariably become connected.

Gräper also lays great stress on his embryo 'C.' In the interpretation of the conditions in this embryo I believe that Gräper is in error, provided one may judge from the appearance of his figure 27. On p. 398 he describes the operated side as follows:

> Die Narbe geht rechts dicht an der Grenze zwischen Area opaca und pellucida hin, so dass der grösste Teil der letzteren erhalten ist. Der hintere Teil des Primitivstreifens wurde vollständig vernichtet, so dass auch am Embryo nur die vordere Hälfte entwickelt ist. . . . . Bei näherer Prüfung der Schnitte, bemerkt man links ein schön entwickeltes Herz (Fig. 29), rechts dagegen eine ähnliche Erweiterung, die mit einem Herz nichts gemein hat (Fig. 28). Das Mesoderm spaltet sich hier typisch in seine beiden Blätter. Die Splanchnopleura aber ist hier im Gegensatz zur anderen Seite und zum normalen Verhalten auch an der Stelle, wo die Herzhälfte zu suchen ist, sehr dünn und legt sich dem Entoderm eng an. Zwischen Entoderm und Splanchnopleura sind auf dieser Seite in der ganzen Serie keine einzelnen Zellen oder Zellenzüge zu finden. Hier sei erwähnt, dass es sich aus der Serie leicht feststellen lässt, dass die betreffende Zellschicht wirklich die Somatopleura ist und nicht, wie es den Anschein erwecken könnte, die Wand eines abnorm erweiterten Gefässes. Durch diese glückliche Experiment wird also der oben erwähnte Einwurf, der gemacht werden könnte, entkräftet. Es ist nämlich sehr wohl die Stelle, wo sich das Herz sonst bildet, und darüber hinaus noch ein grosses Stück Area pellucida vorhanden, dennoch ist aber von einer Herzhälfte nichts zu sehen, weil eben die betreffende Seite der Area opaca, aus der, wie wir oben erörtert haben, das Material für die Gefässbildung stammt, durch die Narbe vollstandig abgetrennt ist.
>
> Dieser Embryo scheint mir besonders die Ansicht Rückerts zu entkräften, denn obgleich die ganze hintere Hälfte des Primitivstreif-

> ens in jungem Stadium vernichtet war, haben sich doch auf der Seite, wo die Area opaca unversehrt blieb, Gefässe und ein Herz gebildet, was nicht hätte geschehen dürfen, wenn jene Ansicht, auf der die Hahnschen Experimente basieren, richtig wäre.

Judging from his figure 27, one may well question whether the 'Narbe' has really excluded the area opaca. A line of separation within the area pellucida has never in my experience yielded such an enormously thick 'Narbe' as that shown in this figure. The tissue on the extreme right of the figure is in all probability a portion of the area opaca. Its failure to develop endothelial cavities (and perhaps to absorb yolk) is difficult to explain. One never finds the splanchnopleuric mesoderm (myocardium) in the heart region a single-layered structure unless there is a pronounced oedema, so that the distension produced by the coelomic fluid enormously stretches the mesodermal walls. This distension may furnish a mechanical explanation of the failure of the cardiac endothelium to form on the side on which the oedematous condition is exhibited. Certain it is that endocardial tissue had little space in which to develop under such conditions. The space between splanchnopleure and entoderm is completely occluded; in other words, if this explanation be correct, endothelium was potentially able to develop if the mechanical relations of the coelom had not prevented it. One could also assume that those conditions which produced the abnormal coelom had likewise injured the tissue which would have produced prevascular anlagen, and that both abnormal conditions resulted from the same cause.

Hahn (17, pp. 410–413) has also commented on the conditions in Gräper's embryo 'C'. He points out the fact that in "Graper's embryo 'A' the heart is also unilateral. He states (p. 410):

> Die Tatsache, dass eine der operierten Seite entsprechende Herzhälfte bei Embryo A nicht zur Entwicklung gelangt ist, begründet nun Gräper damit, dass dieselbe 'eben nicht nötig war.' Er führt dies dann noch weiter aus mit den Worten: 'Auf der operierten Seite wurde die Anlage der Gefässe durch den Eingriff verzögert (also nicht total verhindert!), so dass die andre Seite Zeit hatte, eine Herzhälfte zu bilden, die dem ganzen Herzen gleichwertig war; nunmehr war die Bildung des zweiten Herzens unnötig und unterblieb.'

Hahn calls attention to the fact that if such argument as that of Gräper concerning embryo 'A' were valid it could likewise apply to the conditions in Gräper's embryo 'C,' which is above described. I believe with Hahn that the question of necessity is inapplicable equally in both cases. In both these instances the development of endothelium was 'unmöglich' whether it was 'unnötig' or not. Gräper's explanation of his embryo 'A' would defeat his own theory of multiple hearts, in that only one is 'nötig;' it is inconceivable that all supernumerary heart-anlagen have equal advantages for their own preservation and development. Doubtless in both Gräper's embryos 'A' and 'C' he has simply produced conditions such that one-half the heart anlage was unable to develop—otherwise both would have developed bilateral hearts if the embryos were normal prior to operation. If an endocardial anlage ever started on the operated side in either case, it could in all probability have been detected in these embryos when they were sectioned.

Gräper misinterprets Hahn's conclusions when he states that the development of endothelium in his own embryo 'C' disproves the validity of Hahn's work. So far as I am aware, Hahn did not claim that a destruction of the posterior portion of the primitive streak would inhibit the formation of endothelium.

Gräper's operations were generally performed on such early stages that the orientation of the embryo was uncertain. In some cases his 'posterior' cauterizations were no doubt somewhat lateral.

I am convinced from my own experiments at least, that Gräper's one 'fortunate case,' embryo 'C,' is insufficient to overthrow the view of Rückert and Hahn.

2. The work of Hahn, and Miller and McWhorter

As we have already seen, the work of Hahn was announced by Rückert in Hertwig's Handbuch before Gräper's account was published, but the latter account was published prior to the publication of Hahn's own observations; owing to these circumstances the work of each was known to the other. Their results were quite different; this explains the fact that it is impossible to review each work separately.

Hahn's work (17) covers a number of years of experimentation. His descriptions are concerned with the conditions obtained in seven blastoderms. Like Gräper, he employed the cauterization-method in separating the embryonic body on one side from the blastoderm, and in the destroyal of the primitive-streak mesoderm. In most cases he found that a destruction of the posterior portion of a side of the primitive streak prevented the formation of blood-islands in the blastoderm on that side. In one case (Embryo III, p. 376) he describes a large vesicle on the operated side of the blastoderm, lying near the wall of the wound opposite the embryonic body. This vesicle is filled with fluid and the cells of its lining are flattened out so as to resemble endothelium. He states:

> Es ist nun eine Frage, ob man solche von Flüssigkeit erfüllte, jedoch nie geformte Elemente enthaltende sinus als Gefässanlagen, oder als cytöse Bildungen, die mit typischen Vorgängen nichts zu tun haben, auffassen will. Jedenfalls scheint ihre Bildungsweise mir auf die Fähigkeit des Dotterendoblast hinzuweisen, leere mit teilweiser Endothelauskleidung versehene Lacunen bilden zu können.

Lying below this vesicle was a small isolated group of blood cells, some of which were intimately associated with the lower wall of the vesicle, but none entered it. Hahn considers this case as possibly analogous to some of the " 'aberranten Blutinseln' Gräpers." He states that the emptiness of the vesicle of corpuscles "spricht nicht gerade für ihre Natur als Blutanlagen,' but leaves the question open.

Concerning the origin of the intraembryonic vessels, Hahn's results speak exclusively for a local mesodermal origin. On page 426 he states:

> Wenn ich demgemäss meine eignen Beobachtungen zusammenfasse, so komme ich zu demselben Schlusse wie bezüglich der Herkunft der Aortenzellen, dass nämlich das Material für das Herzenendothel ebenso wie das Aorta 'genetisch unabhängig' ist sowohl von Dotterendoblast wie von dem embryonalen Teil des Darmdrüsenblattes. Die Relation zwischen dem Auftreten einer Herzanlage auf der gestörten Seite einerseits und dem Umfang des erhaltenen Splanchnopleura-

> stückes anderseits, ferner die Beobachtung des Austritts des fraglichen Materials aus diesem Rest des visceralen Mesoblastblattes zwingen mich, eine lokale Entstehung der Endothelelements aus der genannten Quelle zu vertreten.

Two of his experiments are of particular interest. In his embryo I, the posterior end of the dorsal aorta is described as follows:

> Im Bereiche des letzten Ursegments beteilen sich nur mehr ein bis zwei Endothelzellen auf dem Querschnitt an der Bildung der Aortenwand, und fast gleichzeitig mit dem Aufhören des letzten Restes einer Seitenplatte ist auch kurz nach dem Eintritt in die unsegmentierte Rumpfregion die Aorta verschwunden. Erst nach einer Fläche der auf der linken Körperseite restierenden mittleren Keimblattmasse neuerdings erst einzelne, dann zwei bis drei Gefässzellen am Schnitt wieder auf, die auseinanderweichend wiederum ein deutliches Gefässrohr umgrenzen (Fig. 24, Taf. XII).

In his embryo II the posterior locus of origin of the vitelline vein and cardiac endothelium of the operated side is described (p. 402) as follows:

> Diese besteht in einer lokalisierten Auflockerung des hohen mehrzeiligen Epithels des Darmfaserblattes, wodurch es einer Anzahl auffallend spindelförmig ausgezogener Elemente möglich wird, teils isoliert, teils in kleinen Ketten aus dem Epithelverbande herauszutreten und mehr und mehr frei werden. Diese lagern sich in dem Spaltraum zwischen Darmendoblast und visceralen Mesoblast herein und bleiben dabei zunächst mit ihrer Ursprungsstätte noch in Zusammenhang.

Farther anteriorly a lumen appears in the group of cells which may be traced into the heart. The cardiac tube has no connection with that of the normal side. Anteriorly (p. 404):

> . . . . es verengt sich rasch und löst sich in einzelne lateralwärts ziehende Gefässanlagen auf, ohne dass, wie früher erwähnt, es gelingt ein aufsteigendes Bogengefäss und seine Einmündung in die dorsale Aorta zu finden.

Hahn evidently anticipated the possible objection that the normal endothelium might have vascularized the operated side. He gives reasons for believing that such was not the case.

The experiments of Miller and McWhorter (39), like those of Hahn, consisted in the separation of one side (and also one end) of chick embryos from the extraembryonic blastoderm. The conditions which they obtained were very similar to the results of Hahn. Their work contributes two distinct advances over that of Hahn. They reconstructed the endothelial tubes obtained and still more important, their method of separation of the axial portion of the body from the blastoderm was by incision rather than by the crude and uncontrollable method of cauterization or burning. The amount of injury along the line of separation is certainly much smaller in the method introduced by Miller and McWhorter. Their work was submitted as proof of the local origin of endothelium. Prior to, and following its publication the objections urged against this work were inclusive of the following: (1) the incisions may not have been made sufficiently close to the embryonic axis; (2) they may not have been made sufficiently early; (3) endothelium may have grown in from the opposite side or from the ends. Prior to my own work (52) the only published comment on the interpretation of local origin in such experimental cases, besides a short discussion by Schulte is that of Bremer (4, p. 462) who in discussing the work of Gräper, Hahn, Miller and McWhorter, states:

> From the differences in the conclusions reached by two of these authors (Gräper and Hahn) it seems certain that more work should be done along these lines before a consensus of opinion can be expected. I wish to point out a few possibilities which should, I think, be considered in any such future work.
>
> As shown in my reconstructions of rabbit embryos, the vascular net has an irregular mesial border, certain strands lying further toward the midline than the position of the future aorta.
>
> Though in young embryos the extension of these strands across the median line of the embryo proper to form a net on the opposite side is rendered impossible by the close approximation of the medullary groove, notochord, and entoderm, yet long before the stage figured in many instances cited by these authors the mesoderm has grown across the median line and might afford a pathway for endothelial sprouts from side to side.
>
> Another and earlier pathway is at the posterior end of the embryo, behind the primitive streak where the mesoderm very early extends across the median line. The angioblast cords, by which connections from recognizable blood-vessels to apparently isolated angiocysts can be traced (if we accept, for the moment, and for the purpose of argument, the extension theory) are delicate strands, easily overlooked, and

moreover may last only a few hours, if the mechanical conditions are not favorable to their continued development into vessels. It is not to be expected, therefore, that anything short of a very complete series of such operated embryos, fixed at progressively longer intervals of incubation after operation, can settle whether or not there is any extension from the opposite side.

Concerning the communication of vessels of the two sides, I may say that such connections may sometimes be found in the head-region ventral to the pharynx in completely isolated head-meroplasts. Such cases (fig. 22) probably have little significance for the experiments of Hahn, and Miller and McWhorter.

Whatever may have been the validity of the objections urged against these experimental results, the above review gives the exact status of the endothelium-controversy at the time at which the present work was begun. In general my results have been made known through a previous publication (52). These former results, together with many more recent ones are incorporated in the present communication.

IV. MATERIAL, METHODS AND OBSERVATIONS

1. Chick material and mechanical methods

We have considered the previous experimental results on chick material, and have enumerated the objections urged against their finality. Doubtless the most serious objection to the experimental work on chick material which had preceded my own, was that of the possibility of ingrowth from the opposite side. It had been suggested that nothing short of a study of a large number of closely graded stages could furnish a basis for conclusive evidence. It is evident, however, that such a study might still remain open to the objection of those who require still more thorough-going investigations. Such a situation must necessarily confront us unless there be some means of altering the technique of operation, or increasing its severity to an extent which will eliminate the necessity (though by no means, the desirability) of such an extensive study. It occurred to the writer that a feasible method of eliminating the possibility both of ingrowth and of further extension from the opposite side, would be that of isolating both sides from the outlying blastoderm—or better still, to isolate completely the embryonic body or portions of it from all the outlying blastoderm at a time before the embryonic tissue is vascularized. It is evident that if endothelium should then appear in the isolated tissue following further incubation, such endothelium could not possibly represent an extension or ingrowth of a yolk-sac vessel. Under these conditions the question of growth from the opposite side would not concern us, so far as the proof of local origin is involved.

As before noted, it was objected that the operations of Miller and McWhorter may not have been done early enough, and that their incisions may not have been made sufficiently close to the embryonic tissue. In seeking a means of overcoming these objections I happened on the following procedure: at the time of operation it is possible to remove the yolk-sac blastoderm lateral to the line of separation on one or both sides. In case only a head-fragment is to be further incubated, it is possible to remove with the blastoderm that part of the body-axis which normally should first contain blood-vessels. This tissue is preserved at once and sectioned. In this manner one can determine exactly the status of the extraembryonic vascular tissues. By this means it is possible, with safety, to let the embryos develop to a much more advanced stage before operation than would be permissible by the methods of any previous observers. From this it is not to be inferred that in all cases it is desirable to wait until the latest possible time of exclusion of ingrowth before performing the operations. If the general method be trustworthy, one should be able to get positive results long before vascular tissue has developed dangerously near the embryo's body. The great advantage of operating at as late a stage as possible is, that the abnormality produced is likely to be less, and growth and differentiation proceed farther the older the embryo is at the time of operation. If allowed to summarize what I believe to be my contributions to the experimental technique of studying endothelium, I would say that so far as mechanical methods are concerned they are these: complete isolation of the unvascularized tissue, and a means of obtaining accurate knowledge of the conditions in the outlying tissue. Given these, the method becomes a critical one. It should satisfy all reasonable demands of experimental proof. To say the very least, the endothelium which can develop in the isolated tissue under such conditions cannot have grown in from an outside source.

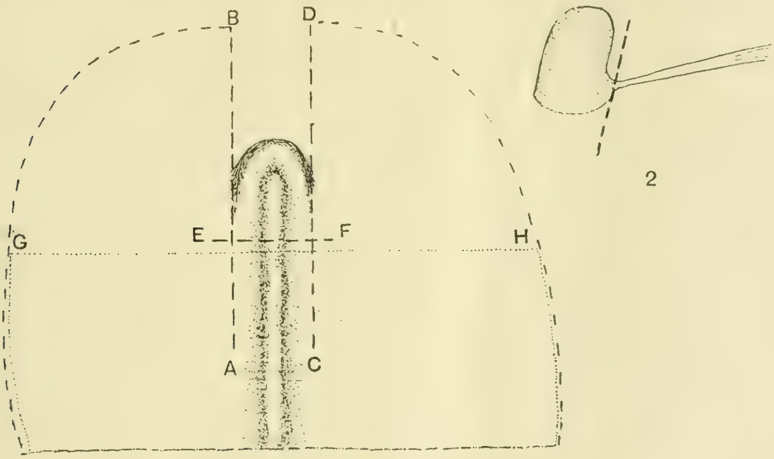
Whether the endothelium which undoubtedly does form under these conditions represents in a faithful manner the essentials of its normal development is not for me to say. All that can be claimed is that my figures show conditions which were actually obtained. Further study alone can determine what significance is to be attached to these conditions.

Record of material used in illustrations in Part I

TYPE	NO.	INTERSOMITIC GROOVES AT TIME OF OPERATION	TOTAL INCUBATION	STAIN	BLASTODERM EXAMINED	ILLUSTRATED IN FIGURES
			hours			
I	19	1	32	m.b.-e.	+	11
I	24	0	29	m.b.-e.	+	12
I	18	1	30	m.b.-e.	+	13
II	3	2	48	m.b.-e.	++	16, 17, 18
I	31	2	32	m.b.-e.	++	14, 19, 21
I	17	1	33	m.b.-e.	+	15
I	122	0	34	m.b.-e.	++	20, 22
I	111	0	31	m.b.-e.	−	29
I	34	0	29	m.b.-e.	−	35, 36
II	133	0	38	Fe.h.	+	23
II	96	3	48	Fe.h.	−	24
II	116	1	40	Fe.h.	+	25, 26, 27
II	52	1	38	Fe.h.	+	28
II	124	2	41	Fe.h.	+	30
II	149	0	36	Fe.h.	+	31, 32, 33
II	53	2	39	Fe.h.	+	34
II	88	2	44	Bx.c.	+	37, 38
II	14	0	35	Fe.h.	+	39, 40, 41, 42, 43,
11	48	0	37	Fe.h.	+	44, 45, 46, 47, 48

Explanation of abbreviations used in table: *m.b.-e.* refers to my modification of Mann's methyl blue-eosin stain, *Fe.h.* indicates iron haematoxylin; *Bx.c.* indicates borax carmine. Two plus-marks indicate that the extraembryonic blastoderm is shown in illustration.

b. Complete isolation of meroplasts. In general the methods of operation have fallen under two types: Experiments designated as Type I are those in which a small portion of the embryonic body was completely isolated from the outlying blastoderm. An examination of figures 1 and 2 will explain the methods by which this complete separation was accomplished. Chick embryos prior to the formation of intersomitic grooves, and stages up to the formation of three intersomitic grooves constitute the material studied. It was found, on examination of the blastoderms that blood vessels were not generally to be found near the embryonic axis at the time of the first intersomitic groove. There are, however, sufficient exceptions to this to make an examination of the blastoderms always desirable. Normal incubation was generally allowed for about eighteen to twenty hours; this had to be varied in different seasons.



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1 Diagram to illustrate the blastodermal incisions in experiments of Types I and II. Broken lines represent the incisions in Type I. Dotted line *G* to *H* indicates the transverse incision in Type II in which incision *E* to *F* is omitted.

2 Lateral view of the head-fragment of Type I pushed back to be severed from the proamnion at point where a broken line intersects.

The Type I operation consisted in this: longitudinal incisions were first made, separating laterally the anterior region of the embryonic axis (fig. 1, *A-B* and *C-D*). Next, a transverse incision (*E-F*) served to sever the anterior portion of the body from the more posterior portion; this incision was usually made at the anterior intestinal portal. At this time, before completely separating the head-fragment from the small band of tissue which held it anteriorly, it was found advisable to remove the main portion of the blastoderm. The method of doing this will be understood by following the broken curved lines from points *B* and *D*, laterally and posteriorly; a transverse incision was then made, joining their posterior extremities. The large piece of blastoderm obtained by this means was preserved at once, to be sectioned later. The head-fragment was then completely isolated by the incision shown in figure 2. This figure represents a side view of the head (represented entirely too large) which has been pushed back so that the incision can be made with greater ease. Such a fragment generally sinks to the bottom of the sub-germinal cavity. Following the operation the egg was sealed and incubated to a total age of from twenty-four to forty-eight hours; after that time, degenerative changes could be seen in the tissues. Differentiation did not usually proceed beyond that of a normal thirty-two hour embryo. Growth, on the other hand, varied greatly. Meroplasts equally differentiated might vary greatly in size. As I have previously noted (52, p. 334) it seems that the embryonic meroplast "possesses an inherent capacity for differentiation which tides it over to a time when heart-pulsation should normally provide a means of tissue-respiration."

After the final incubation the tissue-fragment was removed and preserved in picro-acetic acid, sectioned, and stained. The stain ordinarily employed was my modification (51) of Mann's methyl blue-eosin stain. In a number of cases in which an attempt was made to utilize mitotic figures as a means of determining the origin of the prevascular tissue, the sections were stained in iron-hematoxylin and counterstained with acidulated methyl blue. Ordinarily the sections were cut 4 micra in thickness.

Sections of such fragments displayed surprisingly normal conditions. The neural grooves would often close normally. If the incision *E-F* (fig. 1) were made posterior to the anterior intestinal portal, the flat blastoderm would often concresce to form a tube. The sections often contained coelomic cavities apparently farther anteriorly than one should expect to find in the normal embryo. Above all, such fragments generally contained blood-vessels in various stages of development.

A very typical picture, frequently obtained in head-meroplasts severed at the anterior intestinal portal is that seen in figure 11. Here in the fore-brain region the neural folds have failed to meet each other. A head-coelom has developed. On the right side of the figure there is a rather large endothelial cavity with a smaller one below it. Such structures are absent on the opposite side in this particular plane of section.

Since some of the figures of this paper have been previously described (52) they need not be dwelt on at great length. Without giving a great deal of evidence concerning the origin of the earliest prevascular cells, I stated (52, p. 334) that "between the base of the coelomic 'pouch' and the pharyngeal entoderm, rounded or cuboidal cells become proliferated. Their point of origin is in most cases between the base of the coelomic 'pouch' and the pharyngeal entoderm where it is difficult to determine which of these two epithelia is of primary importance in such cell-proliferation." The cells in question are shown in figure 12. In figure 13, it will be seen that ventral and lateral to the pharynx such cells have arranged themselves into a parenchymatous complex which merges dorsally into the indifferent mesenchyme. At the time when this figure was first published, I was inclined to believe that such a condition represented an essential stage in the formation of endothelium in this region, and hinted that the resolution of such a complex into endothelial tubes might be closely related to the accumulation of plasma in its meshes. My more recent experiments lead me to believe that such a condition may not necessarily be passed through by the tissue in this region; that the tubes may be formed by a more direct and active manner, in which isolated vesicles are formed without the intervention of a parenchymatous stage; that such isolated vesicles coalesce to form the tubes; that such tubes are enlarged by the addition of single mesenchyme cells, or by the addition of solid or hollow groups of cells. There appear certain cases, however, exhibiting a parenchymatous arrangement of cells which, from their staining reaction, are to be interpreted as true endothelial cells.

In figure 14 is shown a cross (perhaps frontally inclined) section of a head-meroplast. On the right the incision was very close to the brain tube. The dorsal mesenchyme has arranged itself into the form of an epithelium. The incision included the pharynx, causing it to gap open and be displaced by tension of the opposite side. The cut edges of ectoderm and entoderm end blindly. Lateral to the ventrally directed U-shaped portion of the pharynx an endothelial tube is seen on each side. Between these two tubes is a solid ventral connection. The conditions here resemble heart-formation to a certain extent. The coelomic walls have met mesially, but their place of meeting is ventral to the pharynx; the latter has not been constricted for the reason that it was already tubular at the time at which the operation was performed, whereas in heart-formation the transformation of flat entoderm into tubular pharynx is an essential process. A more accurate figure of the ventral region of a neighboring section is shown in figure 21, in which many structures are figured less diagrammatically.

Conditions somewhat similar to these are shown in figure 15. The longitudinal incision, as seen on the left of the figure, was not far from the median plane, and here again the mesenchyme has formed an epithelium which merges so gradually into ectoderm that it is impossible to determine where that layer begins. It will be noted that on the left side of this embryo no endothelium has formed. When the incision is made so close to the median plane, such conditions often result; it seems to stimulate a vigorous growth of mesenchyme which becomes so dense that the formation of endothelium is impossible. Such close incisions are not necessary for the prevention of vascular ingrowth.

A condition similar to those described in embryo Type I, No. 31 (figs. 14 and 21) is seen in Type I, No. 122 (fig. 22). In this embryo the endothelial tubes have a median connection, but in this section their cavities are distinct. Posteriorly the cavities themselves communicate. Despite the great differences in the development of the blastoderms at the time of operation, Type I, No. 31 and Type I, No. 122 show very great similarity of structure; the conditions in the former are shown in figure 19. This figure is a photograph of a cross-section of the blastoderm (exclusive of the meroplast) which was removed at the time of operation. In this operation the incisions *A-B* and *C-D* extended somewhat posterior to the line *E-F*. The plane of section lies between the line *E-F* and an imaginary line *A-C* (fig. 1). Thus we obtain a section showing the body axis, discontinuities corresponding to the longitudinal incisions *A-B* and *C-D*, and lateral to these, the outlying blastoderm. Such a section records both the position of the longitudinal incisions and the conditions of vascular development in the body axis as well as the extra-embryonic area. This section shows the greatest mesial extent of the yolk-sac vascular tissues which are in continuity (fig. 19). Lying here and there, mesial to these vessels, are occasional cells or groups of cells, apparently in process of separating from the splanchnopleure. These may well be regarded as forerunners of vascular tissue. No vessel fulfilling the requirements of a sprout of 'angioblast' had yet approached the median axis. The tissue between the two incisions (fig. 19) represents the middle or anterior part of the hind-brain region. It would perhaps have been the first part of the body to have been 'invaded' by vitelline capillaries, if invasion had been the true process of vascularization. The longitudinal incision represented on the right side of figure 20 was not parallel to the median axis, but approached it anteriorly.

Figure 20 is a photograph of a transverse section of the blastoderm belonging to the meroplast of which figure 22 is a section. The plane of figure 20 is only a short distance behind the line *E-F* of figure 1. The points *A* and *C* in this case lie in the line *E-F*; hence the effects of the longitudinal incisions are not shown in this section. The stage of operation in this case was considerably before the time of appearance of intersomitic grooves. There is as yet no formation of vascular tissues, even in the area opaca. The mesoderm is still uncleft. This and the foregoing case illustrate very well the advantages of removing the outlying blastoderm at the time of operation. One could, with impunity, have allowed this embryo (Type I, No. 122) to incubate longer, provided the blastoderm had been removed to check the result. I have in no case offered as proof, however, any material in which the vascular tissue of the yolk-sac was seen to have come near the line of longitudinal incision.

Figures 29, 35, and 36 are also from completely isolated fragments, illustrating in a positive manner the local formation of endothelium. But in addition to this they display certain special features of interest in other connections to be dealt with later in this work.

c. *Incomplete isolation of meroplasts.* Another type of experiment performed was one which may be designated as Type II. In such experiments the operated tissue was not completely severed from the outlying blastoderm. Generally the embryonic tissue left to further incubation consisted of a portion of the body axis united with the opaque area anteriorly by a small strip of proamnion. It was repeatedly observed that any connection with the blastoderm greatly favored the vitality of the meroplast. Again a consultation of figure 1 will make clear the method of operation. Longitudinal incisions corresponding to *A-B* and *C-D* were made of lengths varying from those which separated only a portion of the head, to those which extended back well into the posterior region. In case only the head or trunk-region was desired, an incision *G-H* served to separate the meroplast posteriorly, and also to separate the posterior blastoderm from the more anterior portion of the body-axis. The blastoderm not included in the meroplast was removed at the time of operation to be sectioned.

In meroplast No. 3 of Type II, the longitudinal incisions reached posteriorly to the plane of the line *G-H*, which also shows approximately the position of the transverse incision which severed the anterior and posterior portions of the embryonic body, and which also was the transverse line of separation of the blastoderm. In this experiment all the blastoderm anterior to the line *G-H* was left for further incubation, while that posterior to it was removed. Figures 16, 17, and 18 show the conditions as seen in sections of the meroplast and its blastoderm-control. Figure 16 is a cross-section through the anterior portion of the fore-brain. The cut edges of the entodermal and ectodermal layers of the proamnion have fused, forming posteriorly a blind sac into which there seems to have grown from the anteriorly lying coelom, a sac which likewise ends blindly within the posterior region of the proamniotic sac. Formerly I regarded the presence of this coelomic sac as abnormal. It is probably a normal structure, so far as the presence of coelom in the proamnion at this stage is concerned. It will be seen that the cut edges of ectoderm and entoderm of the outlying blastoderm have fused. The only possible path by which yolk-sac vessels could have reached the embryonic axis would have been by way of the proamniotic sac. It will be noted that the latter is devoid of blood-vessels.

Figure 17 is a section through the mid-brain region of this same meroplast. Its interpretation will be facilitated by reference again to figure 1. Since the incision *G-H* was made considerably behind the anterior intestinal portal, the axial tissue included between the longitudinal incisions and lying between the anterior intestinal portal and the incision *G-H* was necessarily composed of three layers at the time of operation. Dorsally there was ectoderm, ventrally entoderm, and between the two mesoderm was present. The cut edges of ectoderm and entoderm have fused, and the dorso-ventral expanse of the contained tissue has rounded the axial portion into an almost cylindrical shape. Thus the meroplast has the superficial appearance of a tubular head-portion of the body axis as one finds in the fore-brain region projecting over the blastoderm. Since this section lies behind the anterior intestinal portal, there is no ventrally lying proamniotic sac. Although the body appears to be tubular, it contains no alimentary tube. The ventral wall of the meroplast is really the dorsal wall of the pharynx The anterior axial tissue is very degenerate in appearance. At about the beginning of the mid-brain region there is an extraordinarily abrupt change in the tissue to a very perfectly normal and healthy condition. This experiment yielded the most satisfactory result which I have been able to obtain, due to some favorable condition which I have never been able to duplicate. Two very well developed aortae are present. Figure 18 is a section through the blastoderm of this same embryo. Its plane passes through the hind-brain region where the first axial vessels should be expected. It shows the freedom of the area pellucida from endothelium.

d. Splanchnopleural concrescence and the mechanics of heart-formation. So far we have considered the conditions in the head region where a portion, at least, of the body axis was tubular at the time of operation. It is evident that the region of the body which becomes tubular by the folding and constriction of flat layers must have some interesting experimental possibilities. In the trunk-region the nearness of the longitudinal incisions to the median line profoundly affects the reaction which the tissue will give. If the incisions be relatively near that line the three germ-layers retain their horizontal position, or at least show no tendency towards concrescence (fig. 31; the right sides of figs. 24 and 44). If the incisions be made slightly more lateral (fig. 39) there is a rather abortive attempt at concrescence to produce an alimentary tract. One side will exhibit this tendency, even though the incision on the opposite side was so close to the body-axis that there could be no concrescence (figs. 24, 25, 35, and 44). This shows conclusively that the process is not one of mutual attraction of the opposite sides. Figure 23 shows in a most striking manner the close relation existing between the nearness of incision to the body axis and the degree of concrescence. In this instance the incisions are unequally distant from the median line, and the side containing the greater amount of tissue underwent the greater amount of concrescence. There is almost mathematical exactness in this relation of the amount of intact tissue to the angle of concrescence. Incidentally it might be mentioned here, as will be considered later, that the size of the endothelial tubes obtained (fig. 23) also bears a close relation to the amount of tissue left intact. It will be noted (figs. 23, 25, 30, 38, 39, and 44) that there is a tendency for ectoderm, both layers of mesoderm, and entoderm to remain united at the line of incision. When this happens, as it ordinarily does, the parietal mesoderm and the ectoderm share in this ventrally directed concrescence. Thus in some cases the tubular meroplast produced by the fusion of these ventrally directed complexes may be entirely surrounded by ectoderm (fig. 28) or partly so, as in figure 27, in which a part of the ventral 'body wall' is composed of entoderm. In this latter connection it is profitable to consider again the conditions in figure 17 where, as we have already seen, the entire ventral 'body wall' is entodermal. On the right side of this figure it will be noted that the incision was relatively close to the body axis. Whatever attempt there may have been made on this side at a ventral concrescence was annulled by the remarkable dorso-ventral expansion of the tissues, which even caused the entoderm to bend upwards. On the left of the figure it will be seen that the incision was located somewhat more laterally—obviously more lateral than the most lateral extent of the potential dorsal wall of the pharynx. The apex of the projecting fold on the left side represents the point of fusion of ectoderm and entoderm. This fold is somewhat ventrally directed, so that here we have a feeble attempt at a ventral concrescence. It would undoubtedly be possible to get a completely graded series of trunk-meroplasts from those whose entire ventral wall is entodermal, to those entirely surrounded by ectoderm.

It does not always happen that parietal and visceral mesoderm remain united, as one sees in figure 35; but in all cases this parietal complex tends to be somewhat ventrally directed. In no instance have the parietal layers folded dorsally to simulate amnion-formation. It may be that the mechanical conditions of the operation cause the mesodermal layers to be united for a time, and that when they part, the upper complex is unable to continue a dorsal up-folding. In some cases the parietal layers remain in a practically horizontal position appearing as rather inconspicuous appendages of the visceral lamellae which have evidently continued their growth. This size-difference is shown somewhat in figure 35.

Let us now consider the behavior of the locally formed endothelial tubes during this process. Since in all cases considered the operations were performed at a time before the embryonic fundament was vascularized (as was in most cases actually verified by an examination of the blastoderm themselves) the endothelial tubes in the trunk-region are local formations. Since these endothelial tubes lie in the region of normal heart-formation, their behavior is of interest.

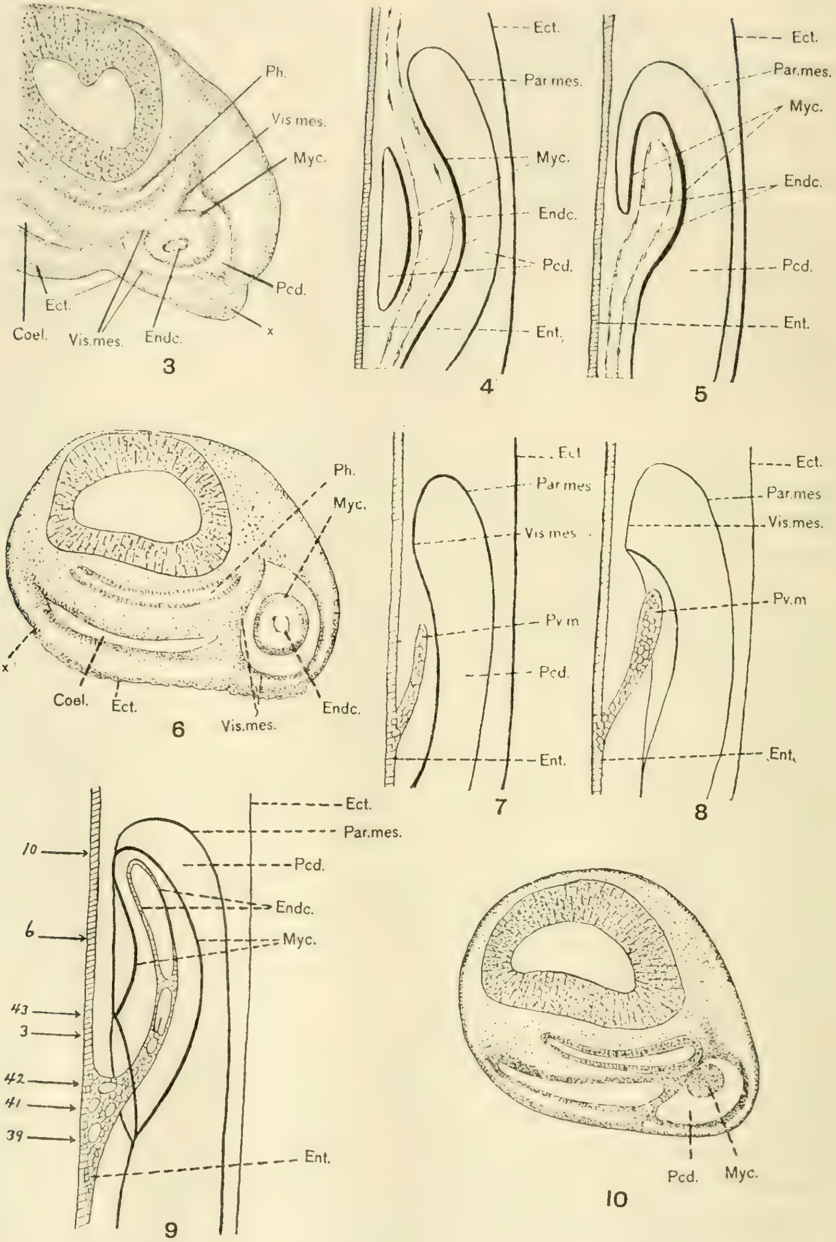
In figure 23, morphologically ventral and internal to the line of incision on each side will be seen in section an endothelial tube. That on the right is the larger, as has already been noted. The adjacent splanchnopleure has invaginated so as to conform to the size and curvature of the surface of the endothelial tube on each side. The conditions remind one of mammalian heart-formation prior to the ventral approach of the bilateral anlagen. It is impossible to say just how far the process would have continued if the tissue had been left to further incubation.

The trunk-meroplast Type II, No. 116 is of interest in that it portrays the process of concrescence as it may be followed progressively from the posterior to the anterior region. Fortunately the incision on the left side (figs. 25 and 26) was nearer to the median line in the posterior region than it was in the anterior region; thus in the posterior region the tissue remaining intact on the left side is much smaller in amount than on the right. As the line of incision on the left side gradually diverged from the median axis anteriorly, the tissue on the two sides became equal by the time the incisions reached as far anteriorly as the tubular head-region. On the left sides of figures 25 and 26 it is seen that the formation of endothelium has been inhibited in the posterior region. Farther anteriorly, however, endothelium has formed on this side so that the median heart in figure 27 is a bilateral structure. Figure 25 shows a well-advanced concrescence. Entoderm can readily be distinguished from ectoderm by the darkly staining globules. In figure 26 the entodermal layers have met and fused. The entoderm which is to form a portion of the ventral 'body wall' of the meroplast (morphologically the entoderm which lies next the yolk) has severed its connection with the tubular pharyngeal entoderm; as there are no endothelial tubes in the immediate region, the opposed splanchnopleuric mesodermal layers have come directly together. Such endothelium as there is lies ventral to their point of contact. Mechanical conditions may account for the failure of endothelium to form between visceral mesoderm and entoderm at a level dorsal to this place of contact. More anteriorly, in sections not shown in these figures, bilateral endothelial tubes fuse mesially to form a single chambered heart (fig. 27). Well-developed dorsal aortae are present. The thickened entoderm on the ventral side of figure 26 is not material derived from the area vasculosa. Its thickness has been contributed to by the vertically lying fused entoderm which has subsided in consequence of the severing of connection of the latter with the tubular pharynx. The place of incision was within the limits of the area pellucida. Also the entoderm from each side was somewhat thickened as this region contains the 'Narbe' caused by each incision. These conditions are not comparable to those of Gräper's figure 24 (16). In my figure 27 the meroplast has a complete ventral 'body wall' which is composed partly of entoderm. In some sections posterior to this, the duplex nature of the heart is indicated by the duplicity of the endocardium; here it is single. One can here detect the double nature of the myocardium by the small, median, ventrally directed point of fusion of the originally right and left splanchnopleural anlagen. Opposite, close to, and directed towards this myocardial protuberance is a sharp projection of the horizontal splanchnic mesoderm (i.e., the mesoderm associated with the 'body wall' entoderm). These two projections represent sectioned folds which are remnants of the ventral mesentery of the heart; they have been continuous structures but have lost connection just as in normal heart formation. The part of the concresced entoderm not utilized in the formation of the pharynx has contributed to the formation of the abnormal 'body wall.' So far as the body wall is concerned, the conditions in this experiment are as if one should remove the yolk of a telolecithal ovum and unite the cut edges of the ectoderm by a splice of splanchnopleure.

Figure 24 is a section through a trunk-meroplast. The incision on the right was rather close to the embryonic axis. On the opposite side the tissue was left in communication with the blastoderm. Concrescence from the left side has not only reached, but has crossed the median line. The large heart is really unilateral, exhibiting conditions closely resembling Gräper's figure 21. This tendency of the unilateral heart to assume the dimensions of a normal heart is often very striking in embryos in which one side is left in communication with the blastoderm.

e. A possible interpretation of certain 'mesothelial funnels' and multiple hearts. One of the most striking phenomena to be observed in meroplasts containing the heart-region is the reaction of visceral mesoderm to the development of endothelial tubes. One is impressed by the fact that where the vitelline veins enter the body axis the visceral mesoderm is profoundly affected. A most interesting problem is the determination of the causal relationships involved. At present it is possible to offer no more than a suggestion for the solution of this problem. This much is certain: in the heart region of these meroplasts excised on each side from the blastoderm, groups of cells become proliferated between entoderm and splanchnic mesoderm; these cells have a tendency to align themselves into solid cords or hollow tubes, sometimes reticular or branched, sometimes singly and sometimes separately as independent structures; correlated with the presence of these structures there is a marked tendency of the adjacent splanchnic mesoderm to attempt to enfold them. Thus if a cord or tube lie longitudinally along the pharyngeal wall, there is a tendency for the adjacent visceral mesoderm to extend a longitudinal fold dorsal to the cord or tube, and a longitudinal fold ventral to it, so that in section there appears a funnel-shaped reflection of the splanchnic mesoderm towards the dorsal surface of the endothelial tube, and a similar one ventral to it. In case there be more than one endothelial tube, one 'funnel' may project between two tubes (figs. 28 and 29). It is rare indeed to find the apex of such a 'funnel' abutting against or pointing towards the middle convexity of the surface of such a tube. These mesodermal folds have a tendency to project into a plane mesial to the endothelial tube to meet each other, thus completely engulfing the endothelial tube (fig. 3). The internal dorso-mesial element of the upper 'funnel' may fuse with the internal ventro-mesial element of the lower 'funnel;' at the same time the external ventro-lateral element of the dorsal 'funnel' may fuse with the external dorso-lateral element of the ventral 'funnel.' When these fusions have taken place (fig. 43) the process may be carried still further. The layer of mesoderm engulfing the tube may separate from the remaining mesoderm at the raphè of fusion of the apices of the two mesial folds or 'funnels,' so that the endothelial tube is now (fig. 4) completely surrounded by a cylinder of mesoderm which is in every way comparable to a myocardium except that there is neither a dorsal nor a ventral mesentery. What corresponds to one lateral half of the normal dorsal and ventral mesenteries now lies mesial to the unilateral heart as a sheet of mesoderm lying parallel to and closely against the entoderm; anteriorly and posteriorly this mesial surface of the unilateral cardiac tube necessarily joins this layer of mesoderm if the cardiac tube again re-enters the body-axis (fig. 4); also the remaining surface of the myocardium is reflected back as parietal mesoderm.

The preceding explanation applies to the condition in which the unilateral myocardial tube 'leaves' the pericardial cavity and joins again the axial portion of the embryo. I have repeatedly found such conditions. Let us now consider a case in which the cardiac tube ends blindly in a unilateral pericardial cavity. Experiment Type II, No. 14 illustrates such a condition (figs. 3, 5, 9, 10, 39, 41, 42, and 43). The results obtained by this experiment can perhaps be explained by imagining the follow-



3–10 Explanation of abbreviations. *Coel.*, coelom; *Ect.*, ectoderm; *Endc.*, endocardium; *Ent.*, entoderm; *Myc.*, myocardium; *Par. mes.*, parietal mesoderm; *Pcd.*, pericardium; *Ph.*, pharynx; *Pv.m.*, prevascular mesoderm; *Vis. mes.*, visceral mesoderm; *x*, point of fusion of ectoderm and entoderm.

ing process to have taken place: in the anterior potential heart-region the entoderm was probably in close contact with the pharyngeal entoderm as illustrated in figure 7. Such a condition may have obtained as is illustrated in figure 7, which is a hypothetical dorsal view diagram of an early stage in the development of this meroplast. In figure 10 the splanchnic mesoderm is closely applied anteriorly to the wall of the pharynx. Anteriorly no pre-endothelial tissue is shown, just as in figure 7. In the posterior heart-region of figure 7 the splanchnic mesoderm lies farther away from the pharyngeal wall, leaving a space into which pre-endothelial tissue was proliferated. Such a condition must have existed in the posterior region of the cardiac coelom, otherwise the conditions in later development could not be explained. As a possible transition between the stage just described and the conditions actually observed in figures 3, 6, 10, and 39 to 44, figure 8 is submitted. The plug of vascular tissue has increased in length and has enlarged somewhat anteriorly. The visceral mesoderm is giving off a mesially directed fold dorsal to the pre-vascular proliferation, and a similar fold ventral to it. At the same time the visceral mesoderm is invaginating to receive the proliferation. Whatever may have been the actual process involved, figure 9 shows diagrammatically the condition found when the tissue was sectioned; the plane of each section is roughly indicated by arrows in this figure. The blindly ending cardiac endothelium is enclosed by a blind sac of myocardium which projects anteriorly into the coelom. The conditions in the diagram are greatly simplified, and no attempt was made to show the conditions accurately. In this, as in many other instances, there was a number of ridges on the inner myocardial surface in addition to the two principal folds which encircled the endocardial tissue. These ridges are characteristic of cases in which the formation of the vascular tissue is somewhat irregular or considerably arrested. Detailed descriptions of the actual sections are given in another connection.

To summarize the facts just considered, it seems that cardiac endothelium can develop when visceral mesoderm does not press too closely to the entoderm. When the endothelium starts to develop, the visceral mesoderm gives off mesially directed folds, and perhaps at the same time invaginates in such a manner as to conform to the contour of the endothelial tubes and to engulf the latter by the mesial fusion of these folds. Such funnels, in the heart region at least, are probably not concerned with the production of endothelium, but rather in furnishing it with a myocardial covering. The covering may be found around a single tubular unilateral anlage; but in case the anlage be branched, or a formation from venous rootlets, the branches or rootlets may be provided individually with a myocardial covering. Such formations are probably in process in figures 28 and 29. Here it will be seen that each endothelial tube is partly surrounded by myocardium. These facts are of interest in connection with the phenomenon of multiple heart-formation which is sometimes observed.

For a number of years, cases of duplex heart-formation have been known, and have been ascribed to various causes. Panum explained double heart-formation on the assumption that a pull on the bifurcated ventral aorta had torn asunder an already formed heart. Rabl and others have attributed it to a failure of the bilateral heart-anlagen to fuse into a single heart. Various cases of multiple hearts have been observed. A most unusual case is that described by Verocay in the proceedings of the Deutsche Pathologische Gesellschaft, 1905. He described a case of a chicken with seven hearts (Heptacardia). At a hotel in Cortina d'Ampezzo, an apparently normal fowl was found on evisceration to contain a peculiar clump of tissue which when further examined was found to contain seven hearts. Unfortunately the specimen was badly mutilated. Later examination of this clump of tissue by Verocay (73) revealed the fact that five of the seven hearts were of normal shape and size. The two which were removed from the rest exhibited normal relationships of heart-cavities, valves, and stems of the arterial vessels. It was impossible to determine the relationships of the great veins and the auricles. Verocay believed that each heart had been provided with a separate pericardium of its own. It is difficult to imagine a process by which such a condition could have been attained. Verocay explained the existence of multiple hearts on the following basis as Rabl and others had previously done: there may have been a failure of the lateral anlagen to fuse; also since each of the lateral heart anlagen normally is a single vitelline vein, it is conceivable that individual hearts had been formed from the rootlets of vitelline veins or from multiple vitelline veins. Gräper points out the fact that in the preparation which Verocay studied there were present several compact knots of liver tissue "which did not represent a mutilated liver, but seemed to be a case of multiplicatis hepatis together with multiplicatis cardis." Such a condition could be explained on the assumption that the venous rootlets or multiple veins had individually been surrounded and invaded by hepatic proliferation from the wall of the gut. Conditions such as these serve to emphasize the importance of the influence of developing parts on each other—a class of phenomena too often overlooked and greatly underestimated.

Gräper attempted to prove experimentally that duplicate hearts result from a failure of the bilateral anlagen to fuse. This he did by a median incision prior to heart-formation. None of the conditions figured by Gräper give a clue to the formation of more than one heart-anlage on each side. My figures 28 and 29 may perhaps be of interest in this connection. None of my experiments has approached the possibility of producing a seven-hearted condition.

f. The dorsal aorta. Meroplasts produced by longitudinal incisions extending well into the posterior region yielded more or less perfectly developed dorsal aortae which could be traced, sometimes continuously, sometimes discontinuously, to the undoubted dorsal aortae of the head-region. Such findings are of importance owing to the fact that it has been regarded as proved by injection (Evans) that the dorsal aortae of the chick are formed by longitudinal fusion of the elements of the mesial border of the vitelline plexus. Figures 30, 31, 32, 33 and 34 show dorsal aortae or aortic anlagen which were certainly not formed from a vitelline plexus, but are local formations. Such aortae are sometimes abnormally small, and sometimes abnormally large. That they are lined with true endothelium can hardly be doubted. Their position is sufficiently constant to assure us of their significance.

g. Endothelium independent of mesothelium. The work of Bremer (4) has revived our interest in the coelom theory of the origin of endothelium. Bremer found that coelomic mesothelium furnishes a source of endothelial tissue in the body-stalk of the early human embryo; he found that the body-stalk vascularized before yolk-sac vessels appear. Here was clearly a case in which the blood-vessels did not represent an ingrowth from the yolk. Bremer made the suggestion that all endothelium might arise from mesothelium. He accounted for the presence of sauropsidan vitelline vessels prior to coelom formation on the yolk by postulating their origin from a "premesothelial stage of mesoderm." I have previously pointed out (52) that Rückert has shown in case of isolated blood-islands, that cell groups proliferated from this early vascular tissue cleave to form slit-like cavities which later unite with other similarly formed cavities to contribute to the formation of the coelom.

In my previous work (52) I attempted to produce self-vascularizing meroplasts from regions devoid of coelomic tissue, but was unable to do so. Since that time it has been found possible to operate sufficiently close to the median line in the posterior region to exclude coelomic mesothelium as such, and yet obtain endothelial cavities of local origin undoubtedly representing aortic endothelium. Such a condition is shown in figure 31. In the experiment which yielded this condition, the incision *E-F* (fig. 1) was made in the posterior region of the primitive streak. The longitudinal incision was just lateral to the mesoblastic somite where the mesoderm was uncleft. Certainly the aorta here could not have come from an epithelial sort of mesoderm; mesothelial origin seems to be excluded. It might be argued that the somite contains cells that are potentially mesothelium, in analogy with the conditions in lower vertebrates. This may very well be admitted. In fact it is possible, in an earlier stage at least, that any of the mesenchyme cells were potentially endothelial, mesothelial, dermal, or connective tissue. The formation of endothelium on the teleost yolk-sac by wandering mesenchyme cells a great distance from mesothelium (77, 50, 66, 53) does not speak for a mesothelial origin of endothelium.

2. The origin of the prevascular mesoderm (mesenchyme)

Thus far, the present account has not been concerned with the origin of the prevascular mesoderm, except with evidence against the view that this mesoderm is an ingrowth from the yolk-sac vessels. It has been noted that Hahn and Gräper each believed himself to have ascertained which of the primitive layers is responsible for this vascular tissue. It has generally been considered by individual authors that one germ-layer, and one alone, is capable of furnishing this tissue. Even those who believe the direct precursor of endothelium to be mesenchyme would probably derive that mesenchyme from one of the germ layers and from no other. Considerations of the supposedly high specificity of endothelium have given rise to this view; a sponsor of such a view would probably maintain that endothelium is such a highly specific substance that its origin from more than one germ-layer is unthinkable. On the other hand it would probably be maintained that mesenchyme is such a heterogeneous tissue that any degree of equipotentiality in mesenchyme of different sources would be equally unthinkable. Considerations such as these probably explain the fact that different germ-layers as producers of the vascular tissue have generally been regarded as mutually exclusive. Yet when one takes into account the ability with which each of these views has been defended it seems possible that each may contain an element of truth. The immediate section of the present discussion is devoted to a possible reconciliation of the two views—namely the mesodermal and the entodermal origin of the prevascular tissue.

a. Endothelium from visceral mesoderm, Let us first consider the posterior axial region. An experimental study as above outlined, if concentrated on the posterior axial region, would probably lead to the conclusion (as did the work of Hahn) that mesoderm is the one source of the vascular tissue. Hahn figured a number of cases in which the splanchnic mesoderm was giving rise to ventrally directed cells or cell-groups in different stages up to their transformation into endothelium. Such cases were more numerous and striking in the posterior region; he believed that the same process was involved in the formation of endocardium. Certainly in the posterior region there can be little doubt that such mesodermal proliferation takes place. Here the dorsal surface of the entodermal layer is generally perfectly smooth, seldom having its basement membrane interrupted. In the posterior axial region the incipient endothelial tubes or plugs are in practically all cases from their first appearance more closely associated with the mesoderm than with the entoderm.

It has already been noted that a dorsal aorta formed locally in meroplast Type II, No. 49. Figure 31 shows a section of the posterior axis of this embryo. On the left side the plane of section passes through one of the independent anlagen of the aorta. Anteriorly and posteriorly in the aortic line it is found in numerous places that the mesoderm is proliferating ventrally directed groups of cells, some of which are solid (figs. 32 and 33). The plane of section of figure 32 is much anterior to that of figure 31; it is a higher magnification showing the tissue lying left of the notochord, including the aortic line on that side. The plug of tissue projecting ventrally from the mesoderm is not in continuity with other similar proliferations. Figure 33 shows conditions somewhat comparable to these. This one experiment has yielded all gradations from these solid tissue-plugs to tubular endothelium. Figure 37 shows a series of such downgrowths from the splanchnic mesoderm. The plane of section of figure 38 is far anterior to that of figure 37 and the magnification of the latter is greater. From the splanchnic mesoderm in figure 37 one sees prevascular proliferations in all stages up to the formation of what might formerly have been regarded as angioblastic cords, angiocysts or 'sprouts' from these. On the left side of figure 29 there runs mesially from the small cyst a solid cord of cells which was observed in section to connect with the dorsal aorta; the latter is not shown in this figure. The vascular structures in this figure are not connected with those in figure 38; the two are very far apart in the series. The figures described will doubtless suffice to show the principle involved.

b. Endothelium from parietal mesoderm. It is possible also by experiment to obtain endothelium in a location where it could not have developed from entoderm. An example of such a location is the dorsal surface of the parietal mesoderm. These observations confirm in every way the previous observations of Schulte (63). Here, singly or in groups, cells become dorsally proliferated; before or after losing connection with the mesoderm (figs. 37 and 38) they may acquire cavities, thus forming cysts in every way comparable to those of the splanchnopleure; these cysts may be connected by solid cords of cells or by cytoplasmic filaments. Here we have pre-endothelial tissue completely separated from entoderm by two layers of coelomic mesoderm and by coelomic cavity. There is no observable mesial communication between visceral and parietal vessels, and certainly none by way of the yolk-sac. Obviously we are justified in regarding mesoderm as a possible source of endothelium.

It will be noted in figure 37 that the ectoderm and entoderm have failed to fuse. In instances similar to this I have found that a tube of splanchnic endothelium may grow out laterally until it projects beyond the line of longitudinal incision. This is not only an example of the ability of endothelium to grow, but it is also an illustration of the fact that the direction of growth is not necessarily mesial. In rare cases a vascular tube of the splanchnic variety may send a growth laterally and upwards around the fused mesodermal layers and form a parietal vascular cavity. Actual parietal cavities most often exist, however, which could not have been formed by such a growth, having no connection with the splanchnic vessels, the latter likewise having no connection with yolk-sac vessels.

c. Cardiac endothelium from entoderm? It is a familiar fact that in many forms the endothelium of the heart appears from the first to be closely applied to the pharnygeal wall, often seem-ing in the normal individual to have arisen from entoderm. A most striking case of this sort was reported by Lee (27). It is of importance to know whether intimacy of endothelium and entoderm is of primary or of secondary significance. It is highly probable that the association of the two tissues is in some way causally connected with the fusion of the bilateral endocardial tubes. This might be brought about in one of, or a combination of two ways. First, endothelium of mesodermal origin might approach and become applied to the entoderm at a very early stage in its genesis; second, this intimacy of connection might be a genetic one; it might be that the assumption of the rôle of vascular proliferation is merely a local adaptation for the process of heart-formation in the amniotes. Either of these two processes might better insure the meeting of the endothelial tubes, following the withdrawal of the intervening entoderm. The immediate section of the discussion is devoted to the second possibility.

I shall now present what I believe to be actual instances of entodermal proliferation of prevascular mesoderm. It may be that I have attached entirely too much importance to these apparent cases of endothelial proliferation by entoderm. If this be true, it has been for the purpose of putting to as severe a test as possible the view in favor of which I have at times been prejudiced—namely the view which regards the mesoderm as the only possible source of the prevascular tissue.

In the posterior region where endothelium is unquestionably of mesodermal origin, it was seen that cells were moved bodily from the more or less ragged surface of the mesoderm. When such cells could first be recognized as forerunners of the vascular tissue they were invariably more closely associated with mesoderm than with entoderm. In the middle heart-region this was often observed not to be the case; the vascular cells seemed from the first to be more closely related to the entoderm than to the mesoderm. At the anterior and posterior limits of the heart, transitions between these extreme conditions exist. Now it is not characteristic of entodermal cells to leave their epithelium and wander about as such in the interstitial regions. If cardiac endothelium can come from entoderm, one should be able to find entoderm in the act of proliferating that tissue. Such proliferation might well be expected to exhibit mitotic figures. From these considerations it seemed desirable to stain sections of a number of the meroplasts with iron haematoxylin. Mitotic figures in the tissues were generally found to be very scarce; in one case they were found to be almost incredibly abundant (figs. 40 to 44). In this connection it is interesting to note that in a demonstration of material at the thirty-second session of the American Association of Anatomists, Sabin (61) showed that mitosis in chick blastoderms proceeded by definite rhythms, the actual time of mitosis being of exceedingly short duration. In some of Sabin's preparations, almost every endothelial cell could be seen in almost precisely the same stage of mitosis.

As we have previously noted, Hahn stated that the basement-membrane of the entoderm is never interrupted by a cellular proliferation; thus he considered its proliferation of endothelial tissue to be out of the question. Let us now consider certain cases in which the basement-membrane undoubtedly is interrupted. Instances of this are most often found in the heart-region, such as is shown on the right side of figure 39. Internal to the line of longitudinal incision, the entoderm is seen, even at this low magnification, to be greatly thickened. This figure will serve to locate approximately the same parts more highly magnified in figure 40. Here it will be seen that certain entoderm cells are in active mitosis. The basement-membrane of the entoderm is undoubtedly interrupted in two places. The splanchnic mesoderm is thrown into a number of folds, none of the apices of which is in contact with the tissue which the entoderm seems to be proliferating. Between mesoderm and entoderm are lightly staining strands of a coagulated plasma-like fluid, stained only by the counterstain.

Figure 41 is a section slightly anterior to the plane of figure 40. Slightly separated from the entoderm is a bridge of tissue which can be traced back to the entodermal proliferation of figure 32, Traced still farther anteriorly (fig. 42) this bridge of tissue is still seen to be in continuity with the entoderm. Near the extremity of this projection from the entoderm there is a vacuole in the tissue. To the right of this vacuole the tissue is solid; from this region there can be traced anteriorly through many sections a longitudinally coursing column of cells. Anteriorly this column contains intermittent vacuoles. The splanchnic mesoderm concresces around this column of cells and (figs. 3 and 43) the apices of the folds produced fuse mesial to the column of cells which has here (fig. 3) taken on the undoubted characteristics of early endothelium. In figure 6 the endothelium is considerably flattened, the myocardial tube has severed connection with the splanchnic mesoderm and now lies inside the pericardial cavity, appearing free in section. Anterior to this (figs. 9 and 10) both endocardium and myocardium end abruptly. The gross structure of this formation has already been considered.

Summarizing the conditions just described, we have here a column of cells, the base of which rests on the entoderm and is being actively contributed to by that epithelium. Whether the entire column has increased by the activity of this one growing-point can not be stated with certainty. It has probably increased also by its own growth. Anteriorly this column of cells possesses the characteristics of endothelium and is surrounded by a myocardium; both end abruptly. Whether the portion which is truly endothelial has come from entoderm, the reader may judge for himself. I strongly favor the interpretation that we have here a true entodermal origin of prevascular tissue.

Of the numerous cases observed in which pre-endothelial mesoderm appeared to be given off by entoderm, I shall describe one more. This is the case of meroplast Type II, No. 148. Figure 44 will serve to locate the region more highly magnified in figures 45, 46, 47 and 48. As we have already seen the right incision was very close to the embryonic axis, leaving little mesodermal tissue on that side. On the left side the incision was farther laterad, so that the tissue which remained could undergo concrescence. The plane of this section is through the potential heart-region. In close contact with the entoderm is an endothelial tube with a dorsally projecting solid process. Let us now consider sections of this same general region more highly magnified. Figure 45 is a section considerably anterior to the plane of figure 44. Dorsally there is a projection from the entoderm; it is continuous with certain other structures in this region whose pre-endothelial nature can scarcely be doubted. While mitotic figures are by no means wanting, they are not so numerous as those in the case just considered. Figure 46 is a section through the middle heart-region slightly anterior to the plane of figure 44. In fact the endothelial cavity of figure 46 can be traced discontinuously back to the endothelial cavity of figure 44. It will be noted that the endothelial cavity in figure 46 is joined to the entoderm by a solid cord of cells at the base of which an entodermal cell is dividing. In the posterior heart-region (fig. 47), interesting conditions obtain. Dorsally there is a plug of cells, evidently being given off from the entoderm. There can be no doubt that the basement-membrane is here interrupted. More ventrally there is a similar proliferation. The latter is in contact with, but not continuous with the visceral mesoderm. Still more posteriorly the conditions are of great interest. Here there appears to be a transition between a region where the entoderm seems to be the source of endothelium, and the more posterior region where the mesoderm seems to be responsible for all the endothelium. In figure 48 it will be noted that the basement-membrane of the pharnygeal entoderm is interrupted and that pre-endothelial cells lie in a matrix common with that of the entoderm and also with that of the splanchnic mesoderm. Destroy the entodermal connection with this pre-vascular complex, and one would say that this complex had originated from splanchnic mesoderm. Posterior to this region the basement membrane of the entoderm is intact, and endothelial tissue has no connection with any tissue other than visceral mesoderm.

Figure 35 is a section just anterior to the heart-region of meroplast Type I, No. 34. Such a condition as this would probably be interpreted as an instance of mesodermal origin of endothelium by delamination. This section is more anterior than the region represented by figures 45, 46, 47 and 48; it is the ventral extremity of a left fold comparable to that of figure 44.

Whatever may be the significance of these conditions above described, they demonstrate the fact that projections from the entoderm resembling pre-vascular tissue undoubtedly can be found. In view of their demonstration we have these alternatives of interpretation: either the entoderm is capable of giving rise to vascular tissue, or such proliferations and projections from any tissue whatsoever are to be distrusted as indications of the actual origin of the vascular tissue. If one searches with sufficient diligence, he is likely to become convinced that neither the view of the entodermal origin, nor the view of the mesodermal origin of the vascular tissue embodies the whole truth; conversely, he is likely to be convinced that each view contains an element of truth. The two views are best harmonized by considering the pre-vascular tissue as mesenchyme, whatever may be its source. If cardiac endothelium actually arises from entoderm, the phenomenon may well be considered a mere local adaptation to the circumstance that it is advantageous to have the bilateral endocardial tubes closely approximated in anticipation of their future fusion. The whole question may well remain open, whether one or both layers are capable of giving rise to vascular tissue. It seems highly probably that both layers share in the production of endothelium. Subsequent observation alone can determine the significance which is to be attached to these undoubted conditions which I have actually observed, and have figured as faithfully as possible.

3. The origin of endothelium in hybrid teleosts

Loeb (28) first demonstrated that chemical treatment or hybridization in the teleost would inhibit the development of a blood-circulation, even though the embryo was able to develop a beating heart. Stockard (64) has shown and also Werber (78) has shown in case of chemical treatment, that the lack of circulation is caused by the failure of the independent vascular anlagen to become continuous. McClure (37) has extended these observations to the lymphatics. The effect of hybridization was again observed by Newman (45). Thorington and the writer

(55) have shown that in hybrid embryos of the teleosts the vitality of the embryo is often so low that it is never able to develop endothelium beyond the stage of the independent anlagen. The embryo from which our figures 13 to 15 (pp. 94–96) were taken is of unusual interest, both because of the condition of its endothelium and the position of its blood-cells. Later, Newman[2] states:

> Among the most interesting anomalous conditions seen in these hybrids are the various disturbances in relation to the parts of the vitelline and systemic circulation. The heart and its main vessels frequently appear disjoined from the body and exhibit an independence in differentiation and an automaticity truly striking. Many problems might be cleared up by a study of these conditions.

Incidentally it might be mentioned that my own results obtained from chemical treatment confirm the observations of other observers concerning the origin of endothelium under these conditions.

5. Observations on living teleost material

In the yolk-sac of the chemically treated teleost embryo from which figures 61 and 62 were made, mesenchyme cells were followed in their active migration during their process of transformation into endothelium. Individual cells in some cases aligned themselves into solid columns which later became hollow. Among the more rounded endothelial anlagen a case was observed in which a rather confused group of young blood-cells and intermingled mesenchyme-cells arranged themselves into a vesicle containing blood-cells. Anastomoses between already formed vascular cavities were seen in some cases to be a result of an actual sprouting of the endothelium itself.

In the cardiectomized embryo from which figure 82 was made, the heart was removed while it was a very slender tube. It had never pulsated. The living mesenchyme cells were later followed on the yolk of this embryo. They were migrating from the posterior body-region to the posterior portion of the yolk-sac.

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[2] Newman, H. H., Proc. Am. Soc. Zool., 1915.

All the mesenchyme cells on the yolk which later formed endothelium and blood-cells and chromatophores migrated ventrally and forward beyond a zone comprising the posterior yolk-surface. Thus it is seen that the posterior surface of the yolk is free of endothelium, blood-cells, and of chromatophores. Sections of this embryo show that no endothelial tubes exist in this posterior yolk-region. There is no connection of yolk-sac vessels with those of the embryo's body posteriorly, anteriorly or laterally. The yolk-sac vessels represent a large number of discontinuous spaces with intimately associated chromatophores. In the tail region of living embryos it was possible to follow free mesenchyme cells in the formation of endothelium, though the process was here complicated by the presence of large numbers of mesenchyme cells. These results on teleost endothelium confirm those of Wenckebach, Raffeale, and Stockard.

If allowed to estimate the relative values of mechanical experimentation on chick embryos and chemical treatment or hybridization of teleost embryos, I should say that the former is the more conclusive. In the body of the teleost, it is impossible to say whether discontinuous spaces have always been out of continuity. It is true that there has been observation (66, p. 586) indicating that a vessel once having formed, has little or no tendency to collapse. I was formerly led to believe this to be true. Studies during a subsequent spawning season of Fundulus have convinced me that this question of collapsing vessels will bear further investigation. In regard to the yolk-sac vessels of the teleost, it may be stated that no one has doubted the local formation of yolk-sac vessels in any group of vertebrates.

PART II. THE ORIGIN OF BLOOD CELLS IN TELEOST EMBRYOS

As many writers have observed, the actual genesis of the blood is obscured by the fact that blood cells are swept from their places of origin before their differentiation is completed. By the motion of body fluids, blood-cells become swept into places where they did not originate. As Stockard was perhaps first to suggest, it is evident that if circulation could be prevented without altering the normal process of blood-formation, a great deal of light would be thrown on that normal process. Stockard (55) employed alcoholic treatment as a means of preventing the circulation of body fluids. He found from such study (64, p. 125) that there are "two distinct and limited places of origin" of red blood cells—the stem-vein, and the posterior surface of the yolk-sac. In his writings he repeatedly states in entire paragraphs of italics that if there has been no blood-circulation, the following regions and parts never contain a single erythrocyte in any embryo at any age. These regions include: (1) all the anterior blood-vessels, (2) all the anterior mesenchyme, (3) the anterior yolk-surface, (4) the heart, and (5) the liver. He claims that leucocytes form only in the anterior mesenchyme of non-circulating embryos. Later in the discussion I shall consider Stockard's work in connection with my own; this statement of his results will suffice to show the differences in our findings.

1. Hybrid material

Thorington and I (55) have shown that hybrid teleosts devoid of circulation may develop erythrocytes in practically any region of the body and of the yolk-sac. The embryo from which our figures 13 and 15 were taken is of great interest. The heart was solid at both extremities, yet it contained erythrocytes. Perhaps a blood-lacuna formed as a portion of the heart. The cardiac tube was widely separated from ventral aorta by mesenchyme. The aorta was barren of blood-cells, yet the precardinals contained many erythrocytes, as did the ducts of Cuvier. No blood-cell ever entered the aorta from the cardiac tube. The aorta itself was discontinuous. For further details see the original communication (55).

2. Chemically treated embryos

The following section is devoted to a description of conditions obtained by early chemical treatment of the developing ova of Fundulus heteroclitus. The descriptions are based principally on embryos treated with weak solutions of acetone, butyric acid or potassium cyanide. Generally the ova were placed in these solutions for twenty-four hours or less, and then reared in running sea-water. The results obtained demonstrate that erythrocytes form in the intermediate cell mass and on the posterior yolk; in addition to this, they indicate that erythrocytes may form in any of those locations in which Stockard claims they never form in embryos without circulation. Some of the conditions about to be described have been previously reported (53).

It is a striking fact that under chemical treatment the embryos tend to exhibit abnormalities. Still more striking is the fact that all the tissues in the anterior end seem to be the most seriously affected. It is rare indeed that the circulation can be inhibited without injury to the anterior end of the body. The condition of oedema in the pericardium produced by chemical treatment swells the pericardium, thus stretching the heart until it becomes solid, or until it is unable to propel the blood. A treatment sufficiently rigorous to render the pericardial cavity oedematous is generally sufficient to create marked disturbance in the anterior tissues. By studying a very large number of embryos, however, one is able to find a few cases in which the circulation has been inhibited and yet at the same time the anterior region bears some resemblance to the normal condition. If we concentrate our study on such embryos as these, we arrive at very different results from those of the observer who concentrates on the majority of embryos whose anterior ends have suffered specifically from the abnormal treatment. Whether the anterior end is injured or remains normal, the posterior end of the chemically treated embryo generally is very little deranged. A lethal treatment is often without serious effect on the posterior region. The explanation of this phenomenon is very difficult. It is merely one of the axioms of development which we must recognize. Child (6) deals with this phenomenon under the term 'axial gradient.'

The vascular tissues on the teleost yolk-sac are formed largely from mesenchyme cells which migrate there from the posterior axial region of the embryonic body. Now it generally happens that conditions which will produce oedematous pericardia will also produce embryos with yolk-sacs whose anterior portions lack endothelium and blood-cells. This fact probably finds its explanation in the circumstance that conditions which will prevent circulation will also vitiate these migrating mesenchyme cells to the extent that they are unable to reach the anterior yolk, or are rendered unable to form these vascular tissues even though they reach that location. But here again it is possible to find many exceptional conditions. Many instances can be found in which migrating mesenchyme cells can reach the anterior yolk and there form blood-cells, even though the heart has never produced any circulation whatever. Thus it is that in chemical treatment, especially if the chemical treatment be severe, a large number of embryos are able to develop red blood cells in only those positions where Stockard claims they can develop. There is most often a coincidence between the absence of erythrocytes in the anterior region of the yolk and the anterior axial region. There are, however, many exceptions in which this barrenness of erythrocytes holds good for only one of these regions, even though the blood has not circulated. If an observer, in selecting his material, uses as his sole criterion for the failure of circulation the absence of erythrocytes in these regions, his method is capable of giving only one result.

Examination of the rather small percentage of embryos devoid of circulation which have been able to develop relatively normal eyes and forebrains often reveals in such embryos a tendency to exhibit locally formed and sometimes bilaterally symmetrical patches of erythrocytes at an early stage in the ontogeny. These patches may be in any position with reference to the eyes, and sometimes even in the optic cups. The time during which they exhibit their red color is usually very short. Sometimes following the appearance of a patch of erythrocytes on one side of the head a similar one would appear on the other side symmetrical with the one first observed, the latter having in the meantime lost its color. During the study of such conditions, all possible efforts were made to detect any movement of red blood cells, either active or passive. Such changes would take place in embryos whose hearts were solid. The region mesial to the optic cups, and sometimes the snout, displayed such patches of erythrocytes. Blood sometimes developed in the dilated portions of hearts which were solid at both ends. In some cases the heart had solid extremities, but contained a number of blood-bearing dilations.

Figures 49 to 60 are sketches of the dorsal cephalic surface of living embryos whose blood-circulation was prevented by chemical means. In some of these embryos the heart was solid at one extremity or both, or solid throughout. The stippled areas represent these patches of erythrocytes. Whenever such an area was sectioned and stained, the same group of cells could be located in section, the erythrocytes staining intensely red in my modification of Mann's methyl blue-eosin stain. The surrounding mesenchyme would stain a light or intense blue. In some cases the hematopoetic areas found in section were larger than one should expect from the sketches of the living condition. This probably means that cells may stain intensely red even though they are not visible in the living condition. In no case was the eosinophilous region smaller than the group of erythrocytes seen in the living conditions. The method of chemical treatment is either given in the text with the discussion of each embryo or with the explanation of the figures. Figures 49 to 56 show dorsal views of the cephalic regions; figures 57 and 58 are lateral views. The nature of the heart and the distension of the pericardium can be observed in these figures.

In the embryo from which figure 49 was taken, several blood lacunae could be seen on the dorsal surface. Two such layers are located dorso-mesial and two are dorso-posterior to the eyes; a fifth median group is found dorsally located in about the transverse plane of the posterior limits of the eyes. Each of these groups of cells, together with certain others which were invisible in the living condition, were found when the embryo was sectioned. They are illustrated in figures 67 to 70. The heart of this embryo was, from the first, solid at both ends.

In figure 50 are shown two lacunae dorsal and mesial to the optic cups. Their red color could no longer be distinguished four hours after this figure was made. Figures 51 and 52 are sketches of the dorsal cephalic surface of an embryo the history of which is given in greater detail in a previous publication (53, p. 103). At its four-cell stage the ovum was placed in a solution of 50 cc. sea water containing 10 cc. of $\frac{1}{12}$ molecular butyric acid, where it remained for twenty-four hours. It was then placed in running sea-water. On the ninth day a blood lacuna was observed over the left eye (fig. 51). Sixteen hours later this lacuna could not be seen, but another located symmetrically with its former position was observed on the right side. When the embryo was sectioned, eosinophilous areas corresponding to both these lacunae were found in section. The explanation of these circumstances is rather difficult. It seems unlikely that the hemoglobin should leave a cell unless it should disintegrate. It may be that the overlying mesenchyme rendered the first lacuna invisible. Figures 53 to 56 need not be further discussed. They merely show the position of others of the lacunae which were observed in this anterior region. While the location of the smaller groups, and especially that of the single isolated erythrocytes is rather indefinite, there are certain regions rather characteristic for erythrocyte-formation.

Figure 56 shows a very exceptional condition. No case similar to this was found in the entire series of experiments. In this instance, the posterior portion of the embryo was the part affected; as a result it is diminutive in size. The anterior end is relatively normal. Correlated with these conditions we have discontinuous lines of erythrocytes extending into the ophthalmic region, which were not carried there by heart-pulsation. The rudimentary posterior end contains no erythrocytes.

Figure 58 shows a side view of an embryo which, at the four-cell stage, was treated with a mixture of equal parts of 4 per cent alcohol (sea-water solution) and a solution made from 2 cc. of $\frac{1}{100}$ per cent KCN in 50 cc. sea-water. The embryo developed without a circulation. The heart possessed solid extremities. On the twelfth day a longitudinal column of erythrocytes could be observed through each translucent otocyst. Within the next three days a single median hematopoetic area was observed in the mandible. These conditions continued for some time. Figure 58 shows the conditions on the twenty-fifth day. Blood cells were observed apparently scattered on the floor of the pericardium. Later sectioning of this embryo indicated that the erythrocytes on the floor of the pericardium had formed locally beneath the mesothelium.

It sometimes happens that embryos develop without forming a heart; sometimes there is little, if any embryonic body present. In such cases the entire yolk-surface may be covered with blood cells, diffusely scattered or in groups; such a condition may obtain when there is practically no endothelial tissue present. Such conditions furnish a positive disproval of the claim that blood cells never form on the anterior surface of the yolk if there has been no circulation. Figure 59 shows a condition in which a small portion of the body axis abuts on a coelomic cavity which appears to be a pericardial cavity. No heart was ever formed in this embryo, yet no region of the yolk was free from blood cells. In figure 60 a condition is represented in which no embryonic body ever developed. No particular region of the yolk, even from the earliest stages following chemical treatment, seemed especially entitled to be designated as embryonic body. The blood cells were somewhat scattered, but in some cases they were densely crowded. In the center of the region towards the observer, a blood-mass is surrounded by endothelium.

A very unusual condition was observed in the embryo from which figures 69 and 70 were made. At its four-cell stage this embryo was treated for twenty-four hours with a 3.5 per cent solution of alcohol. On the sixth day a bright red blood-lacuna was observed on the antero-ventral surface of the yolk. No other erythrocytes could be seen on the yolk or in the body axis at this time, though mesenchyme cells in abundance were migrating on the yolk. There was no very extensive system of endothelial tubes. The heart was apparently normal, but its venous end had not connected with any yolk-sac vessel. On the tenth day the blood-lacuna on the anterior yolk, somewhat elongated, displayed waves of contraction which started in the end nearer the heart and proceeded ventrally to the opposite end. Evidently this lacuna was provided with some sort of myocardium. It had developed a dorsally directed flexure, as seen in figure 69; the yolk endothelium is not shown in this sketch. At this time the churning of the heart could be seen to loosen the closely packed cells in the intermediate cell mass which had begun to acquire a faint orange color. On the fifteenth day a careful examination of the posterior region of this embryo revealed the fact that certain erythrocytes were being dislocated by the movement of the heart, and some could be followed to a large lacuna on the yolk, although there was no complete circulation of the blood. Such cells would oscillate slightly from the motion of body-fluids caused by heart-pulsation. Blood lacunae on the yolk may sometimes be of this sort of formation. The formation of the endothelial tubes and cavities on the yolk was watched in the living condition. As has already been seen, at the age of ten days this activated lacuna, accessory heart, or whatever it may have been, was pulsating feebly. On the nineteenth day (fig. 70) the lacuna or 'accessory heart' was pulsating seventy times per minute, while the real heart, seemingly normal in shape, was pulsating one hundred times per minute; the latter was in no way connected with any yolk-sac vessel. Likewise no endothelial tissue had ever approached, or formed near the accessory heart. The yolk-circulation was established through the normal heart on the twenty-fifth day. At that time the rate of heart-pulsation had greatly increased, especially in the accessory heart. Rather suddenly on the twenty-fifth day the rate of beat in the accessory heart increased to 136 times per minute while that of the normal heart was 126 times per minute. The accessory heart increased its rate somewhat, and then began to decline considerably on the twenty-sixth day, when its rate became slower than that of the normal heart. At this time the embryo was killed. The flexure of the accessory heart had greatly increased. It is difficult to explain the extraordinary manner in which the rate of beat of the accessory heart was changed. It is conceivable that we here have to do with the failure of an inhibiting nerve-supply to its musculature. Unfortunately I was unable to section this embryo, as it was in some way mislaid. This misfortune was in a measure compensated for by the fact that the observations on this embryo were made under circumstances (The Marine Biological Laboratory, Woods Hole, Massachusetts) in which I could avail myself of the counsel of many investigators, a number of whom were so kind as to examine the embryo in question and verify my observations. At the time at which the sketch for figure 69 was made, the embryo was examined by Profs. E. G. Conklin, H. McE. Knower, E. V. Cowdry, E. I. Werber and many others. At a time somewhat later after the yolk-sac circulation had been established, the embryo was examined by Prof. C. R. Stockard. This embryo furnishes an example of a completely isolated functional heart containing blood-cells.

Attention is now called to the conditions in one of the most important embryos obtained from the entire study. In this fortunate case the conditions which were able absolutely to prevent the formation of vascular tissues on the anterior yolk, and to cause the venous end of the heart to be solid, did not prevent the formation of erythrocytes in the anterior vessels or in the anterior mesenchyme. This embryo is also to be considered presently in connection with the formation of blood in the aorta and the liver. It seems certain that mesenchyme cells in these various parts were locally transformed into erythrocytes. On the left side of figures 62 and 63 will be noticed a cup-like invagination of the fore-brain tissue resembling closely an optic cup. In the place in which one should expect to find a lens, there developed at an early stage a conspicuously red blood-lacuna. No other endothelium or erythrocytes could be found in connection with this lacuna, either in the living or sectioned material. Dorsal to the optic tissue on the right side of figure 55 are a few erythrocytes which were found lying in the crevices between the peculiar folds of nervous tissue. Some of the cells in these locations seemed to be transforming into erythrocytes, but bore unmistakable traces of a mesenchymal nature. Such isolated cells were found entirely out of relation to all endothelium.

The heart of this embryo (fig. 63) is seen to be solid in its anterior portion. More posteriorly (fig. 62) in the arterial end the endothelium has a lumen. The endothelium again becomes solid immediately at the 'insertion' of the arterial end of the heart on the embryonic body. A section passing through the posterior limits of the diencephalon shows well developed precardinal veins (fig. 64) so closely packed with erythrocytes that the latter have angular contours. Still more posteriorly in the region of the otocysts (fig. 75) the precardinal lines are seen to contain erythrocytes. This section also passes through the very small portion of the ventral aorta which was able to form. Almost immediately posterior to this plane of section, the aorta completely disappears. Besides this small portion of ventral aorta, no portion of the branchial arterial system was found in section. The remainder of the ventral aorta, the aortic arches and cephalic dorsal aortae are entirely absent. Since no vascular tissues developed in the anterior yolk, and no erythrocytes were formed in the heart of this embryo it seems reasonable to assume that these erythrocytes were local formations in the ventral aorta. They may or may not have been contributed to by aortic endothelium.

This embryo received closer observation than most of the embryos studied, for the reason that its peculiar eye-formation rendered it conspicuous even before the blood could possibly have circulated. While a number of mesenchyme cells reached the anterior yolk, no formation of endothelium or erythrocytes took place in this region. This fact was noticed in the living condition and is verified in section. Some of the mesenchyme cells on the anterior yolk would probably have transformed into erythrocytes if the embryo had been allowed to live longer; some of them, still typically stellate, were found to be very slightly eosinophilous. In figure 62 the yolk sac is devoid of vascular tissues. The same is true of all sections anterior to this, and of many which are posterior. Figure 75 shows the most anterior extent of erythrocytes that were found on this yolk-sac. These were far dorso-lateral, and belong to an isolated lacuna.

In this embryo the solid distal end of a very feeble heart ends blindly on a yolk sac the anterior portion of which is, and has always been devoid of vascular tissues. Yet the anterior mesenchyme and the anterior vessels contain blood cells. As will be shown later, the liver of this embryo contains erythrocytes, though these may possibly be mesenchyme cells pushed out with and included in the liver diverticulum.

The conditions observed in figure 49 may now be considered in section (figs. 65 and 66). In each case the position of the blood-producing area is illustrated by solid black dots, while in the illustrations of these regions more highly magnified the erythrocytes are shown by unstippled cells. It is quite impossible to do justice to the preparation by means of one-color illustrations. The erythrocytes stained a brilliant red, the mesenchyme cells a rich blue, while the probable transitional stages between the two stained purple. The erythrocytes seem to be identical in nature with those in the intermediate cell-mass. Figure 65 shows a section taken just posterior to the optic vesicles. The erythrocytes in this figure are not surrounded by endothelium; there is a large group on the left side, and two groups on the right. The latter are shown in greater magnification in figure 67, where instead of the arbitrary black dots we have unstippled cells representing erythrocytes. Some of the cells surrounding this group of erythrocyte groups seem to be flattening out to form endothelium.

In figure 66 we have a section through the dorso-median blood-anlage observed in figure 49. These erythrocytes (fig. 68) lie in a crevice in the fore-brain tissue; they are not surrounded by endothelium, but are bounded entirely by ectoderm. No endothelium developed in any region near this group of cells. It is even doubtful whether one should find endothelial tubes in this region in the normal embryo at a stage corresponding to this.

It is of interest to note that Professor McClure, working with the fresh-water teleost Erimyzon sucetta oblongus, has obtained results similar to those above described for Fundulus. He obtained chemically treated embryos without circulation, having lacunae of erythrocytes in the anterior mesenchyme. His experiments, in fact, preceded my own, but his results were unknown to me until my return from the Woods Hole laboratories. He has kindly placed at my disposal an embryo on which he made daily observation, and which he sketched in the living condition. In the living embryo a group of erythrocytes was observed dorsal to each eye. A section through that region is shown in figure 76. On the left side the erythrocyte group is the more prominent. It is not surrounded by a distinct endothelium. In sections of this region, such crescent-shaped groups of erythrocytes over the eyes are not of rare occurrence. They are found sufficiently often to indicate that the mesenchyme of this region may be a true erythrocyte-anlage. On consulting Werber's figure 24 (78) it will be seen that he found a blood-anlage very similarly located. He describes the blood cells in this region as leucocytes "evidently of polymorphonuclear variety," subscribing at that time to the view of Stockard that the blood cells produced in the anterior region are leucocytes. I regard it doubtful that these are really leucocytes. Be this as it may, the homologous region of the mesenchyme which in Werber's figure seemed to give rise to leucocytes gave rise to erythrocytes in the experiment performed by Professor McClure. It will be seen in figure 77 that the arterial portion of the heart is solid; sections show this embryo to be devoid of ventral aorta, aortic arches, and anterior dorsal aorta. Examination of sections somewhat posterior to the eyes reveals a region posterior to the erythrocytes on both sides of figure 76, which contains no haemal vascular tissue whatever (fig. 78). Thus we see that the vascular anlagen anterior to this are completely isolated. A section through one of the nasal pits of this embryo (fig. 79) shows a small vascular cavity containing a few erythrocytes. The endothelium of this cavity narrows down to an exceedingly fine tube which can be traced back to the region of the vascular anlage on the same side in figure 77. The tissue in this embryo is in a remarkable state of preservation. It was stained in Delafield's haematoxylin and orange G. The differentiation of the erythrocytes is not very great in this stain, so that they might easily be overlooked. In photograph they stand out more clearly than to the eye; this was accomplished by the use of color-screens.

Let us now consider certain embryos devoid of circulation which developed erythrocytes in their hearts. In these instances the embryos were watched carefully at short intervals. It is quite unlikely that a heart, partly solid, would hollow out between these intervals only to become solid again at such intervals. If a blood-cell be found in the heart of an embryo in which there had never been a circulation, there are at least the following possibilities of its origin: The fully formed erythrocyte may have reached that location (1) by its own independent power of locomotion, (2) its mesenchymatous anlage might have reached that location by active migration, (3) the erythrocyte in question might be a transformation of an endocardial cell, (4) the erythrocyte in question might be a transformation of a cell that would ordinarily have become a myocardial cell, (5) it might be a transformed mesothelial cell. These seem to include most of the possibilities. It is evident that if an investigator should state that in the absence of circulation the heart "never contains a single erythrocyte in any embryo at any age" he would relinquish all right to claim that an erythrocyte found in that location had reached it by any of the five possibilities just outlined. A claim that a blood cell never develops in a given location excludes the possibility of claiming at the same time that a blood-anlage had migrated into that location.

The conditions in the heart of the embryo from which figure 49 was taken is of interest. No circulation could be observed in this embryo. On the fifth day it was noted that both extremities of the heart were solid, though it appeared otherwise to be normal. Previous to this time the embryo had received frequent examination. The material seemed very favorable, owing to the fact that its condition seemed unusually near the normal, considering the fact that there was no circulation. The pericardial cavity was not greatly oedematous, and the heart was not stretched. In fact it possessed a well-developed flexure. The venous end of the heart was undoubtedly solid, and no yolk-sac endothelium approached it. A means suggested itself of preventing any elusive sort of circulation which might take place between the intervals of time at which any single investigator could reasonably be expected to examine this embryo. If the heart could be severed, such a circulation would be precluded. A very fine needle was used to sever the heart at its middle portion. The cut ends fused laterally, the fusion being intimate in some places and loose in others. In figure 66 the solid distal portion of the venous end of the heart is shown ventral to the head. On the right side of a section of the heart of this same embryo (fig. 65) taken more posteriorly there will be seen a continuation of the venous portion seen in figure 66; this right portion, followed posteriorly, disappears abruptly. The left portion of this same section pursued posteriorly is found to be the arterial end of the heart which enters the embryonic body; but if the left portion of the heart of figure 65 be traced anteriorly it disappears abruptly, as did the venous portion when traced posteriorly. The two portions are everywhere in the region of fusion well separated mesially.

Figure 71 is a section taken slightly posterior to that of figure 65, near the plane in which the venous portion ends abruptly. In the venous side there are seen to be numerous erythrocytes apparently in all stages of differentiation. Some of them have large nuclei and stained intensely red; others with medium-sized nuclei stain purple; still other rounded cells with small nuclei stain blue. It will be noticed that there is no endothelium and also no myocardium in this portion of the heart, whereas erythrocytes are plentiful. On the left side, no erythrocytes are seen in section; a few scattered erythrocytes can, however, be found in this arterial portion nearer to the embryonic body. It will be noticed that the endocardium in this portion of the heart exhibits a cuboidal form very unlike the normal squamous epithelium. Conditions such as these are often found in these chemically treated embryos. Undoubtedly this is an abnormal condition, but it must have some significance.

So far no attempt has been made to give the source of the erythrocytes found in the heart. The conditions in the heart of the embryo from which figure 55 is taken may be of interest as affording a possible clue to the origin of such erythrocytes; there may be those who would be willing to consider these conditions as evidence that cardiac endothelium can form erythrocytes. If it had been possible to anticipate the unusual importance of this embryo its history would have been kept more fully. With a number of others which had received like treatment this embryo was segregated to a dish from which all embryos were removed as soon as they were observed to develop a circulation. Later it was removed to a dish in which embryos were placed only on condition that their otherwise normal endocardia should be solid at both extremities. Thus it is certain that this embryo once possessed a complete endocardium which was solid at both ends and dilated in the middle. On the eleventh day the upper end of the heart was seen to display developing blood-cells which at first were orange-colored. This color gradually deepened into red by the fourteenth day, when the embryo was killed. Sections of the heart reveal very interesting conditions. Figure 72 shows a section through the distal (venous) end of the heart not far from the point at which the endothelium is solid. The endothelium is here of a cuboidal type similar to that in figure 71. It stained purple. Inside the endothelium are a number of erythrocytes which stained bright red. Outside the endothelial cells lie the myocardial cells which stained pale blue. The plane of section in figure 73 passes through a flexed portion of the cardiac tube. Some of the erythrocytes in this section lie in a column of erythrocytes which can be traced back to those in figure 72. It will be seen that the endocardium is interrupted. Some of its cells are undoubtedly transforming into erythrocytes. At the right of the lower extremity of the V-shaped remnant of the endocardium on the left of the figure there are abundant transition stages between endothelium and erythrocytes. From the main central mass of erythrocytes in this figure there appears in section a rather sharp 'projection.' In this it is easy to follow the contour of what probably was once the endocardium. The cells at the apex of this projection stained a reddish purple. In the middle of this figure it is seen also that some of the myocardial cells have also undergone this transformation, especially in the upper part of the figure. In figure 74 which is still more proximal is shown a region in which both endocardium and myocardium have completely transformed into erythrocytes; the entire content of the mesothelial covering consists of strongly eosinophilous cells of a typical erythrocyte character. It is a region in which endothelium has been observed in the living condition in this embryo.

3. *Hematopoesis in the liver*

Stockard believes himself to have demonstrated that liver tissue is incapable of forming blood cells, and that it has no hematopoetic function in the bony fishes. His conclusions are based on the fact that he failed to find blood cells in the livers of chemically treated embryos which developed without a circulation. If this conclusion necessarily follows, one might claim that the actual finding of erythrocytes in the liver of an embryo which had not had a circulation would prove the liver to be a blood-producer.

One question which should be settled is this: are embryos devoid of circulation capable of developing livers to a stage in which their potentialities are given a fair test? Even though the chemically treated embryo may retain its life for a time equal to that in which the normal liver appears to produce blood-cells, it is questionable whether the conditions in the chemically treated embryo can ever approach the normal condition.

If a blood cell should develop locally in the liver it might be of mesodermal or of entodermal origin. The vertebrate liver is morphologically a growth into the ventral mesentery; consequently it has a covering of mesothelium and of mesenchyme. The liver of Fundulus is a growth of the gut into the mesenchyme which in turn is covered by mesothelium. These mesodermal tissues are properly considered a portion of the liver tissue. If any of the mesodermal elements of the liver should turn into blood cells, such transformations must necessarily be reckoned with in the study of the liver. It is conceivable that hematopoesis in the liver might also consist in a direct transformation of entodermal cells into blood-cells.

There seems to be abundant evidence that mesenchyme cells caught up in the liver tissue are capable, just as is true of other mesenchyme cells, of turning into erythrocytes. This alone does not render the liver a 'hematopoetic organ.' Applied to any structure which embryos devoid of circulation are capable of developing, the term 'hematopoetic organ' is probably a misnomer. Figure 81 shows a section of the liver diverticulum. A part of the tissue in this section lies near the junction of the diverticulum with the gut. The erythrocytes which developed in the periphery of this liver may have come from mesenchyme cells captured by the outpushing entodermal tissue. This figure is from the embryo of which figures 62, 63, 64 and 75 represent sections. Reasons have already been given for the belief that this embryo never developed a circulation. The erythrocytes in figure 81 are neither surrounded by endothelium nor dangerously near any endothelium. It is impossible again to illustrate adequately the differentiations in these tissues by the use of one color. The erythrocytes are brilliant red, the liver tissue is grayish purple, and the mesenchyme is pale blue. Within the liver are certain large cells more coarsely granular than the surrounding cells; their nuclei are larger than those of the surrounding liver-cells, and somewhat eosinophilous. The nuclei of the adjacent liver cells are dense bluish black. The cytoplasm of these larger cells has assumed a purplish pink color. Some of these cells, or at least some of the cells located near them, approach rather closely the erythrocyte characteristics. In some sections of this liver it is impossible to tell whether the erythrocytes present have developed from cells similar to these, or from mesenchymal inclusions.

Figure 80 shows a section through the liver of the embryo of Erimyzon from which figures 76 to 79 were also taken. Lying promiscuously in the liver tissue are differentiating erythrocytes which are out of relation to all endothelium. It seems improbable that the partly solid heart of this embryo could have caused a sufficient negative pressure to have moved these erythrocytes to their present location from another region of the embryo.

Numerous other cases were observed in which here and there a captured mesenchyme cell had probably changed into an erythrocyte. It is likely that the results of chemical treatment tell us little more concerning the potentialities of the fully developed liver than they tell us of the potentialities of spleen and bone-marrow.

4. Ova subjected to low temperatures

Another means of preventing blood-circulation in Fundulus is to subject the ovum to low temperatures at a very early stage of development. This method of developmental arrest is not new. Four-celled ova were kept at about 4°C. for periods of time ranging from twenty-four to seventy-two hours. Development under these conditions proceeded rather slowly. Embryos often developed without circulation. In all probability the lowering of the temperature causes a blastolysis (Werber, 79). The yolk-sac in such embryos is often greatly shrunken away from the chorion, and evidence of egg-extrusions can often be found in the subchorionic space. Embryos so treated often fail to develop hearts. It may be that the region later to produce the heart-anlage in such cases becomes blastolyzed. At any rate, these embryos devoid of hearts are of interest owing to the fact that their anterior mesenchyme is sometimes hematopoetic. I have obtained erythrocytes in such embryos in positions similar to those shown in figures 50, 54, and 55.

5. Cardiectomized embryos

Removal of the heart prior to heart-pulsation is the ideal means by which one may assure himself that no elusive circulation will falsify the picture obtained in the study of blood-development. The heart region may be destroyed even before a heart-anlage can be found. If this be done the question of passive movement of the blood-elements can be eliminated. The operated embryos may be placed in any position with reference to gravity, and it is possible to obtain embryos from which body-movements can not be elicited. The method has other advantages. The only unfavorable chemical effect is produced by the lack of tissue nourishment and tissue respiration, and the accumulation of katabolic materials together with the possibility of infection. Unfortunately these are not always insignificant. The rate of mortality from such operations is very high indeed, probably because of infection. But once an embryo is able to recover somewhat from the operation it furnishes valuable material for study. In chemical treatment the specific ill-effects of the chemical used are to be added to inanition, the lack of tissue-respiration and the accumulation of katabolic products. Furthermore in case of cardiectomy the embryos may in some instances be allowed to develop normally for seventy hours before operation is necessary to prevent circulation, while in chemical treatment the entire development for the first one to three days must take place in a very abnormal environment. But the one characteristic in common with the results of both sorts of treatment is that the anterior region of the body always suffers the most. It seems practically impossible to obtain cardiectomized embryos whose anterior tissues are perfectly normal, though their posterior regions are generally little injured. In some cases a very small portion of the head was severed from the rest of the body and from the heart; it was hoped that the mesenchyme in such meroplasts would be able to form erythrocytes. It was found, however, that these head-fragments would not increase in size. Thus it was found later that the diameter of such fragments was always much smaller than that of the remainder of the body (figs. 84 and 85). The mesenchyme in these small fragments always became very closely packed, so that it is not surprising that blood-cells were unable to develop under these conditions.

There is reason to believe that in some instances the conditions obtained by cardiectomy may represent more faithfully the normal process than can be obtained by chemical treatment. The former method is capable of producing stages more advanced in differentiation and growth than one can obtain by chemical treatment which will prevent circulation. If differentiation can proceed further in cardiectomized embryos, it is not unreasonable to believe that the process there is more nearly normal. It is practically certain that the normal process of hematopoesis is not duplicated in either case of abnormal treatment. So far, the most anterior erythrocytes obtained in cardiectomized embryos are located ventro-mesial to the anterior portions of the otocysts. None has yet been found in the ophthalmic region and in the snout. These experiments were not begun, however, until late in the spawning season of Fundulus. Fifteen hundred cardiectomies were performed with only partial success, so that it is impossible to predict at present the possibilities of this method of experiment. A very few embryos survived the operations. Those embryos which fail to withstand such treatment generally die soon after the operation. In most of the survivors, erythrocytes could be seen in the living condition much anterior to the pectoral fins. Most of the present descriptions are based on the living conditions. So far, very few of such embryos have been sectioned. Thus it is conceivable that some of the embryos now preserved may exhibit erythrocytes farther anteriorly than have yet been figured in case of cardiectomy. Only three of the embryos will be described at present.

Figure 82 shows the living condition of a nine-day embryo, the heart of which was removed when the embryo was sixty hours old. The heart had not yet pulsated. Following the operation the embryo was kept in a moist-chamber in a cool place. The tail of the embryo became rather long and curved over the dorsal body-surface. The embryo could not be provoked to reflex-responses, and never exhibited any movement. The pectoral fins were relatively huge, working in unison with a quivering fan-like movement. Very well differentiated striated muscle developed at their bases. It will be noted that the head is relatively small; at the anterior limits of the otocysts it narrows down so that its diameter in the region of the eyes, as seen from the dorsal side, is less than half that of the region of the otocysts. The eyes are merely rudimentary optic stalks. There are neither optic cups nor lenses. The pericardium is oedematous. It contains no trace of a heart except an exceedingly minute vestige of its arterial 'insertion' which can be seen only in section. The erythrocytes visible in the living condition are conventionally represented by means of black dots. A column of erythrocytes formed in the intermediate cell-mass. Endothelial cavities, many of which were surrounded by pigment cells, are represented as dark ramifications on the yolk. There are no continuous channels. Large numbers of erythrocytes developed in the immediate region of the pericardium, and some developed beneath its mesothelial lining; no erythrocytes or endothelium developed on the posterior yolk-surface. Sections of this embryo reveal no connection of systemic and yolk-sac vessels. Ducts of Cuvier, as well as vitelline veins, are absent. The posterior yolk is seen in section to be quite free of vascular tissue. Owing to the translucency of the otocysts it was possible to locate the columnar hematopoetic region mesial to each otocyst. A section through this region (fig. 83) shows the position of these cells ventral to the mesial portion of each otocyst. Traced posteriorly these columns disappear, having absolutely no relation to the intermediate cell-mass. The brain tissue is somewhat abnormal. The pharynx and cartilaginous gill-bars are well developed. The abnormal filamentous processes from the pharynx contain numerous darkly staining spherical bodies which when examined at a higher magnification are found to contain small darkly staining globules. These bodies resemble yolk globules somewhat in their staining reaction. In abnormal cases these bodies often become imbedded in the mesenchyme, and might be wrongly interpreted as leucocytes or lymphocytes. Ventral aorta and aortic arches are entirely absent. Dorsal aorta is encountered only in a region much posterior to this.

Figure 84 shows an embryo in which the heart-anlage was destroyed before it could be distinguished. At the same time a small portion of the head was isolated. At this time, optic vesicles could not be distinguished. An eye developed from the fragment of fore-brain tissue which remained. The otocysts were destroyed. There is no trace of a heart. Erythrocytes developed discontinuously in the intermediate cell-mass. Projecting diagonally forward and upward in the median plane from the postero-dorsal portion of the yolk is a slender group of erythrocytes, the nature of which has not yet been determined, as the embryo has not yet been sectioned. It is certain that these cells are not a part of the intermediate cell-mass as such. It will be noted that the dorsal portion of the trunk contains pigment. The clear region ventral to this is filled with a clear fluid. The pectoral fins are well developed. The mesenchyme anterior to the pectoral fin is hematopoetic. On the posterior yolk are a few large blood-lacunae. Some smaller ones are found near the diminutive pericardium. The region between these two blood-bearing areas of the yolk does not contain endothelial tubes. The intersomitic grooves are distinguishable in this embryo; such is rarely if ever the case in chemically treated embryos.

Rather unusual conditions were obtained in another cardiectomy (fig. 85). Before a pericardial cavity had been formed, and before heart-formation had taken place a very fine needle was introduced ventral to the head-tissue. Not only was the future heart-tissue destroyed, but the needle was then manipulated so as to sever the body axis in the mid-brain region. The needle did not puncture the body-wall ectoderm. A large spherical oedematous space probably corresponding to the pericardium has formed; instead of forming anterior and ventral to the head-fragment it has formed behind the latter, so that the head fragment has been pushed considerably forward. The diameter of the isolated cephalic tissue has increased very little beyond that at the time of operation, whereas the main part of the body has grown a great deal. The otocysts were partly destroyed, and the remains of them have probably moved forward somewhat. There was no heart-formation. Erythrocytes were observed ventral to the otocysts and anterior to the pectoral fins. An area probably indicating the point of emergence of the ducts of Cuvier from the embryonic body contains erythrocytes. The intermediate cell-mass is hematopoetic. The yolk-sac contains numerous endothelial vesicles and pigment cells, but no erythrocytes were detected on it in the living condition.

6. Transplanted meroplasts

It has been seen from Stockard's work and my own that chemical treatment will often produce embryos, the anterior and ventral portions of whose yolk-sacs are practically free from endothelium. These embryos may at the same time have a slight dorso-lateral circulation through the ducts of Cuvier and the dorso-lateral yolk-capillaries. A number of such embryos were selected. To the endothelium-free area it was possible in a few cases to transplant an anterior fragment of a normal embryo which had not yet had a circulation. The procedure was as follows: the chorion of the 'host' was partly removed over the endothelium-free area. If the rotation of the yolk-sac became troublesome, it was possible to prick the latter in some region where the injury would not be serious. The slight extrusion would hold the embryo in place. A very slight abrasion was sometimes made on the yolk-area where the fragment was to be transplanted. A small receptacle for the tissue was then placed in a wide-mouthed bottle provided with a cork perforated to permit the intake and outlet of gases. Oxygen was then generated and allowed to replace the greater quantity of air contained in the bottle; the latter was then sealed. The tissue was moistened from time to time with sea-water which had been boiled and cooled. The oxygen supply was renewed at such times. On the whole the experiment was quite unsatisfactory. The tissue would generally either die or become invaded by endothelium.

We may consider, however, the most successful attempt which was made. The procedure was similar to that above described. The anterior region, including the otocysts, was transplanted so its ventral surface rested against the exposed yolk-area of the 'host.' The anterior end of the meroplast was directed anteriorly. The transplanted tissue was badly mutilated, although all possible precautions were taken.

Figure 86 shows a section of the transplanted tissue and its position on the yolk. The morphologically dorsal surface of the tissue lies toward the lower left hand corner of the figure. If the figure be rotated until this corner becomes uppermost, one can orient himself with reference to figure 87, which is the same section of the meroplast somewhat enlarged. The orientation of figure 88 is similar to that of this figure. Examination of figure 87 shows that the plane of section is through an otocyst, probably the left. There is a very thin nervous epithelium partly surrounded by a cartilaginous otic capsule. Laterally located is evidently the anlage of a semi-circular canal. Strangely enough the ganglionic mass accompanying this otocyst lies lateral to it. The tissue on the right side of the otocyst is a rather dense mesenchyme, some of the cells of which are spindle-shaped and massed into rather fibrous complexes. This region to the right of the otocyst represents the median axis of the transplanted tissue. This section contains neither endothelial tissue nor corpuscles. This is true of the remaining posterior portion of the meroplast. The other figures shown are from planes anterior to this.

It will be noted that the yolk-mesoderm 'ventral' to this meroplast has become fibrous. Higher magnification of this tissue (fig. 87) reveals the fact that there are numerous muscle fibers. These seem entirely comparable to those always observed ventral to the anterior portion of the normal embryonic body. It seems that the presence of this anterior axial tissue brings about the formation of muscle fibers. These are, in all probability, *in situ* differentiations of the yolk-sac mesoderm and not migrations from the mesoderm of the transplanted tissue. This point is difficult to prove.

The most anterior section figured may now be considered. The plane of section (fig. 88) is near the anterior extremity of the meroplast. The axial portion is solid cartilage. To the left of this the tissue contains some ganglionic material and mesenchyme, which lies anterior to the otocyst region of figure 87. Within the next few sections anterior, this region on the left entirely disappears, leaving the cartilaginous axis alone in section. The region between the solid cartilage of figure 87 and the axial portion of figure 88 contains mesenchyme cells differentiating into erythrocytes. The cells are rather scattered and none of their groups is large. Figure 89 shows a section taken between the planes of figures 87 and 88. The mesenchyme stained clear blue while the erythrocytes stained red. Intervening stages (*Erthbl.*) take a purple stain. It seems altogether improbable that these erythrocytes have reached their present location through the circulation. The only blood-vessel which could be seen to approach this meroplast was a spur which projected towards its anterior extremity, but never reached it. Also the hematopoetic area is separated from the vessel by solid cartilage. The posterior region of the meroplast contains no vascular tissue. The transplanted tissue is bounded ventrally by a compact sheet of muscle.

Now this region of the yolk to which the meroplast was transplanted is one of those regions in which the mesoderm, according to Stockard, is incapable of forming blood-cells. It is one of those "regions in which wandering blood anlagen never make themselves manifest." If there was no circulation of the blood, the erythrocytes must have differentiated locally. But this mesenchyme is cephalic, and cephalic mesenchyme, according to Stockard, can not form erythrocytes. The reader may judge for himself which (if either) of these claims of Stockard seems questionable in view of the conditions here described.

The embryo from which the meroplast was removed was preserved at the time of operation and sectioned to make sure that none of the intermediate cell-mass had been transplanted. The posterior end of the meroplast proliferated a great deal of tissue which evidently induced a very active migration of mesenchyme from the posterior region of the 'host.' The region between the two contains a considerable amount of tissue of a nondescript sort.

By no means is an attempt made to estimate the value of this experiment for the solution of the question at hand. The work of transplanation has not been carried far enough to demonstrate its true worth. Greater experience in the manipulation of this method might lead to results which would be truly important. The main trouble lies in the extremely low viability of the isolated tissue.

II. GENERAL CONCLUSIONS

1. Does abnormal treatment obscure the normal process?

At this point I wish to consider certain phases of Stockard's work, taking up first his results, and then the conclusions which he drew from them. In commenting on his previous work (65) he stated (66, p. 583) that this work seemed "in the light of past literature to render highly probable, if not to prove, the polyphyletic origin of the various types of blood-cells," thus disposing of the "now extremely improbable monophyletic view." He regards his technique of study as so highly satisfactory that "the only disadvantage is that the worker may be led to wonder whether so apparently a simple problem is of scientific importance." He believes himself to have disproved the monophyletic view by demonstrating that erythrocytes of the teleost arise only in the intermediate cell-mass and on the posterior yolk; that leucocytes originate only in the anterior end of the body; that erythrocytes can not develop from endothelium, from anterior mesenchyme, in anterior vessels, on the anterior and ventral yolk and in the liver.

The first question which every one must decide for himself is, whether he will accept the introductory statement of Stockard (65, p. 234) that "it can not be argued, so far as the blood anlage is concerned, that the conditions recorded are pathological or other than those which would occur in the normal genesis of the blood except that it never circulates." If it be true that such argument is impossible, then the whole question is settled once for all. There would seem to be a possibility that it can be argued, and perhaps even proved that the blood anlage has shared in the afflictions which are evident in all the other tissues in Stockard's figures. What would exempt the blood anlagen from the ill-effects demonstrable in the other tissues? These are questions which one should answer before proceeding to other considerations.

From Stockard's own experiments and the experiments of many other observers, it is evident that the anterior end of the embryo is particularly susceptible to the action of chemicals or to any condition which impairs metabolism. Child (6) has shown that here the rate of metabolism is particularly high. It is very common to find the anterior end greatly retarded or highly abnormal while the posterior end of the embryo may be practically normal. Certainly we may say of the nervous, muscular, skeletal, and alimentary tissues that the characteristics which they exhibit under chemical treatment (see many of Stockard's figures) would give a rather imperfect idea of the normal process. The anterior tissues may be so badly upset as to be scarcely recognizable, while the posterior tissues may approach the normal. The investigator who maintains that it can not be argued that the conditions recorded by Stockard are abnormal 'so far as the blood anlage is concerned' must prove that blood is such a stable tissue as to be able to withstand these ill-effects and develop normally, regardless of the conditions which influence so profoundly the other tissues, especially the anterior tissues. A comparison of Stockard's figures 10 and 11 (65) is particularly appropriate at this time.

Stockard's interpretations are based upon the proposition that if under unfavorable conditions erythrocytes can differentiate in one place in the body, all other regions failing to produce erythrocytes under these same conditions are demonstrably powerless to produce erythrocytes under any conditions whatever. Such a view does not permit of different degrees of hematopoetic tendencies for different regions at a given time; it is claimed that any region which would ever produce blood cells at all must necessarily produce them under the given abnormal condition. In other words, if erythrocytes form at all they must necessarily form in all places capable of producing them at any time under normal conditions. From the fact that endothelium grown in an alcoholic environment was able to produce no erythrocytes, Stockard (65, p. 276) maintains that "one is warranted in making the bold assertion that the endothelial lining of the heart and aorta is perfectly incapable of giving rise to any type of blood-cell." As a matter of fact it has never been maintained that endothelium is an important source of erythrocytes as compared with many other blood-producing tissues. The relatively few cases of this sort are generally regarded as significant principally for the evidence which they afford of the non-specificity of endothelium.

It is conceivable that a tissue whose hematopoetic tendencies are weak would be the first to be affected by abnormal treatment. Stockard reasons somewhat differently. He states that "if vascular endothelium had such power, then one might expect that this power would show itself where it is most needed, for example in these embryos in which the blood never circulates." One might take the equally teleological, but opposite view that these embryos are the very ones which do not need erythrocytes. Stockard goes still farther and maintains (65, p. 311) that the alcoholic environment should be able to stimulate endothelium to form blood cells, provided endothelium is capable of being so stimulated. He points out that no endothelial cell has ever been seen to give rise to a blood cell. Should this observation ever be made, it becomes of interest to know whether the phenomenon would be regarded as adequate proof of the ability of endothelium to form blood, or whether it might be regarded as an instance in which the blood anlage was 'exotic' (65, p. 395) or one in which the process of development had not been clean-cut (65, p. 292).

Concerning the following points it is difficult to determine definitely the interpretation to be placed on Stockard's statements. He figures leucocytes in very young stages, yet (65, p. 309) regards their late appearance as significant. He states (65, p. 280) that they have never been observed except in the anterior region of young embryos, yet believes (65, p. 284) that they are of a wandering disposition, later making their way into all parts of the embryo. Rather strangely, on page 269 (65) he states that "in no case has any type of lymphocyte or leucocyte been present in these yolk-islands except as late wandering cells," whereas he repeatedly states that yolk-islands are always devoid of such structures in the absence of circulation. He regards endothelial cells equally specific with blood, and far removed genetically from connective tissue cells and blood cells; he classes endothelial cells with these latter derivatives as being equally specific with pancreatic cells and liver cells (65, p. 323). He assures us that an invisible destiny shapes the ends of pre-endothelial cells, yet on page 316 (65) he states that the mesenchyme "may be admitted to form endothelium largely as a response to physical conditions." He states that erythroblasts never divide when inside an endothelial cavity, and that red blood cells are always formed in or budded into a vascular space; both these statements are probably incorrect.

There are some interesting conditions in Stockard's own material which he seems to have misinterpreted. Let us examine first his basis of belief that no anterior vessel ever contains a red blood cell if the blood never circulates. We must first agree on what is meant by, and included among the anterior vessels. Doubtless the pre-cardinal veins may be regarded as anterior vessels; doubtless also the ducts of Cuvier would serve as a place of demarcation between anterior and posterior vessels. In all of Stockard's figures and discussions the terms 'precardinal,' 'duct of Cuvier' and 'post-cardinal' are entirely wanting, so far as I am aware. No term more definite than cardinal vein or stem-vein is used. In the normal individual Stockard misinterpreted the ducts of Cuvier as vitelline veins. He figured vitelline veins arising between the otocysts. If we admit that the adult teleost has a duct of Cuvier, as is generally believed, then the 'vitelline vein' figured by Stockard are ducts of Cuvier whose sources between the otocysts are the precardinal vein. The term 'vitelline vein' is correctly used to distinguish particular yolk-sac vessels of a very definite morphology. Thus in the normal individual, Stockard misinterpreted the ducts of Cuvier, while in most of his experiments its development was prevented, so that in neither case was he able to utilize the only satisfactory landmark by which to determine the anterior vessels. No doubt the embryos in many of Stockard's figures have erythrocytes in their precardinals. Figures from Wenckebach[3] and especially those of McClure (36) demonstrate the fact that in young stages the teleostean duct of Cuvier is well posterior to the otocyst. See also Reagan and Thorington (55), figure 15. The 'splitting' which Stockard often found in the anterior portion of the 'stem vein' probably represents precardinal veins. The 'cardinal vein' of Stockard's figure 9 (65) is almost certainly a duct of Cuvier with its anterior continuation, the precardinal.

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[3] Hertwig's Handbuch, p. 1131.

At the thirty-second session of the American Association of Anatomists, the writer presented evidence that erythrocytes may develop locally in many regions in which Stockard claims they never form. The objection was immediately raised that the red blood cells observed in these positions had migrated. Such objections had been anticipated (53, p. 116) and answered as follows: "I am not at present concerned with the possibility of those cells having migrated from some other position; the present situation is relieved of such perplexing considerations when Stockard goes so far as to say that the heart, anterior vessels, anterior yolk, and liver are regions in which wandering blood anlagen never make themselves manifest. He also speaks of erythrocytes 'originating' on the yolk when it is certain that their ultimate anlagen did not occupy that position."

Thus it is seen that blood cells are described as 'originating' (64, p. 125) on the yolk, although their anlagen migrated there from some other position. Have we not the same privilege even if it should be demonstrated that cell-migration is responsible for anterior blood cells? Is it reasonable to suppose that erythrocytes should migrate, when in chemically treated embryos which are perfectly normal "so far as the blood anlage is concerned" the leucocytes and lymphocytes of Stockard are *unable to move* from the head regions? The differentiation of a mesenchymal derivative on the teleost yolk-sac in the absence of circulation necessitates migration owing to the original absence of mesenchyme on the yolk. On the other hand, the mesenchyme is so widely distributed throughout the embryo's body that it is not necessary to assume that a blood-producing mesenchyme cell had migrated to a given place within the embryo's body from some other region. Mesenchyme cells undoubtedly can migrate, but the process of migration in case of intraembryonic hematopoesis would scarcely suggest itself except in support of a thesis. It has not yet occurred to anyone to claim that the endothelial cells forming in practically all regions of the body have migrated there from other sources or from any single source. If, however, it should be shown that such migration of erythrocyte-forming mesenchyme within the body is a process of importance in the normal development which Stockard's experimental conditions have inhibited, the demonstration of this fact is of importance. Our ultimate aim is an understanding of the normal process. If unfortunately it be true that chemical treatment is not a panacea for our perplexities concerning the normal processes of vascular development, it is desirable that we realize that fact.

Let us now consider certain of the deductions made by Stockard from his results which are said to render the monophyletic view extremely improbable. If my analysis be correct, his entire philosophy of vascular development rests on the following assertion, in favor of which his own work offers no proof. On page 229 (65) he makes this statement which serves as his point of departure: "There can be no doubt of the great genetic difference between blood-cells and connective tissue cells, yet their parent cells are with our present methods indistinguishable." As a matter of fact the doubt which has been entertained and which still exists as to the great genetic difference of blood and connective tissue is the basis of the very problem with which we are concerned. In the next sentence following the one just quoted it is stated: "We may with equal justice go further and hold likewise that the cells from which vascular endothelium, red blood cells and white blood cells arise are mesenchymal cells really differing in nature according to whether they will give rise to one or the other of the three types." This statement is dependent on the one preceding in which we are asked to accept from authority that preformation follows as a matter of course since the end-results are different. On pages 315–316 (65) we have this same idea carried out still farther. Stockard states:

> This invisible difference determines the destiny of the cell to form either leucocytes or erythrocytes, but we can not stop just at this point; we must go back to the actual beginning. Then it is found that

> although two wandering mesenchyme cells on the yolk of the fish embryo are indistinguishable so far as observations go, yet they are fundamentally different since one is destined to form an erythroblast while the other possesses no such power and can only form an endothelial lining cell or pigment cell as the case may be. This is all the diphyletic or polyphyletic school would ask. . . . . Here we logically stop, for this is what is conceived by embryologists to be an anlage. . . . . To stop with the tissue anlage we find strong evidence that certain mesenchymal cells are designated to form erythroblasts, others leucoblasts, and still others, and these are more universally scattered throughout the body, give rise to vascular endothelium.

On page 283 (65) Stockard states that the "mesenchymal cell, if taken early enough could no doubt give rise to other mesenchymal cells which would later form these types of cells."

Thus it would seem that our 'conceived' anlage is not a very tangible thing. We could not definitely locate such an anlage and be sure that it might not be a mesenchyme cell which might give rise to other mesenchyme cells 'of these various types.' Its existence is assured by authority and not by demonstration. Some place in the morphologically indifferent stage which we can not locate, 'we find strong evidence' that certain mesenchyme cells are designated to this or that fate. But if it be true that 'here we logically stop' (i.e., looking retrospectively), the converse must be true that looking prospectively, 'here we logically start.' We have selected an instant in which the cell-complex appears by all known means of observation to be indifferent, but our intuition tells us that differences exist. Prior to this time none of the diverse progeny could be said to have an anlage. Now if it be the goal of the polyphyletic school to prove that with actual differences once given, the remainder of the process is, in general, one of greater divergence, their goal is easily reached. Doubtless no one would oppose them. True enough the word 'polyphyletic' etymologically conveys the idea of 'many branches.' If this idea has merely to do with the fate of the parts subsequent to the instant at which we 'logically start,' —if in other words our phyleticism has nothing to do with the origin of the parts beyond the point at which we 'logically stopped,' then the general process of development is unconditionally polyphyletic. If it were not so, the process of differentiation would never proceed beyond the point at which our intuition tells us that differences exist, though we can not see them. It would seem that "all the polyphyletic school would ask" (65, p. 283) and again that all the polyphyletic school would demand is the privilege of reasoning in a circle.

The attempt has often been made to prove that two tissues could never have come from a common anlage for the reason that when differentiation has taken place the products of differentiation have different appearances. Thus Clark (9) believed himself to have proved that endothelium could not develop from mesenchyme for the reason that the nucleoli and certain other fine structures of the differentiated tissues were different. This finding should not be surprising if the grosser structures themselves were so different as to render possible the distinguishing of the two tissues.[4]

If one were to neglect the transitional stages from mesenchyme into cartilage he might convince himself that the latter could never have come from the former. But should he recognize these transitional stages he might then claim that transformation took place because the transforming mesenchyme cells were destined to that fate and were therefore specific. Yet is there no other alternative? The work of Lewis[5] and my own work[6] has shown that in one case at least, preformation need not be assumed; it was shown that otic cartilage arises in response to the presence of a sensory epithelium. The work of Burr[7] has since shown the same to be true in case of nasal cartilage. Lewis showed that if the otocyst of an anuran be transplanted to the mesenchyme of a urodele, there developed a cartilaginous capsule typically urodelan in character. Here would seem to be a case in which the realized outcome was surely not an expression of a predestination. If, on the other hand, otic capsules normally were in the habit of developing in practically all parts of the embryonic mesenchyme, such an experiment would have little significance; in that case, all that could be said is that there would be no especial reason for the belief that the formation of the otic capsule was a matter of predestination; preformation in this instance could neither be proved nor disproved. Suppose for instance that some substance were discovered which would cause the formation of endothelium wherever that substance might be introduced into the mesenchyme. If it be admitted that there are mesenchyme cells in practically all regions which are capable of forming endothelium, it would obviously be impossible to say whether the endothelium which had resulted from the introduction of the substance came from mesenchyme cells whose fate had previously been sealed, or from truly indifferent cells whose vascular characteristics had actually been initiated and not merely unfolded, awakened, or stimulated.

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[4] In Prentiss's Text-book of Embryology (Saunders Co., 1915), p. 287, a great deal is made of the fact that F. T. Lewis (Am. Jour. Anat., vol. 5, 1905) observed that "lymphatic spaces do not resemble mesenchyma."

[5] Lewis, W. H., Anat. Rec., vol. 1, 1907, p. 143.

[6] Reagan, F. P., Proc. Am. Assn. Anat., Anat. Rec., vol. 9, no. 1.

[7] Jour. Exp. Zoöl., vol. 20, no. 2, 1916.

By common consent we have generally agreed that there is no especial reason for believing that a widely diffused mesenchymal derivative is specific. It is generally agreed that before a tissue can be claimed to be specific it must conform to three conditions: First, it must have a demonstrable and narrowly limited anlage whose position we can predict. Second, this tissue must never be found producing any kind of tissue different from the one for which the anlage is claimed to be specific. Third, the anlage producing this tissue must never produce any other kind of tissue. If a mesenchymal derivative fails to conform to any of these conditions, there is no especial reason for believing it to be a 'specific' tissue.

In his final conclusion (65, p. 323) Stockard states:

> The facts here presented seem to indicate that vascular endothelium, erythrocytes, and leucocytes, although all arise from mesenchyme, are really polyphyletic in origin: that is, each has a different mesenchymal anlage. To make the meaning absolutely clear, I consider both the origin of the liver and pancreas cells a parallel case; both arise from entoderm but each is formed from a distinctly different entodermal anlage, and if one of these anlagen is destroyed the other is powerless to replace it.

So far as endothelium is concerned, the two cases are most certainly not parallel. There is abundant evidence for the belief that endothelium can form locally in the mesenchyme in practically all parts of the germ layer of which it is a constituent. There is no evidence that pancreatic cells form locally in, or even migrate into practically all regions of the entodermal tissue. One can predict, in case of pancreatic and liver tissues, approximately the very region in which the anlagen will make themselves manifest; to say the very least, one can predict that in the greater part of the entoderm no pancreatic or liver anlagen ever exist. This can not be said to be true of mesenchyme cells capable of forming endothelium. If the results outlined in my own work hold good for teleost blood-cells, the development of the latter will likewise be found not to constitute a case comparable to that of the development of pancreas and liver.

The inability of one differentiated tissue to replace or regenerate another has absolutely no bearing on the question. In some species a given organ can be regenerated while in other closely related species that very same organ can not. Obviously a comparison of the regenerative power of different tissues of the same organism or of their ability to replace each other has no importance for the question at hand. The ordinary process is not for a destroyed tissue to be replaced by another equally differentiated tissue, but to be regenerated by a tissue intermediate between the two and more primitive than either.

The monophyletic view does not hold that development shall be chaotic and without system. For the monophyletic view it is not necessary that the diverse mesenchymal derivatives should invariably be found in close association. If it be true that there is really an equipotential stage in which the fate of a mesenchyme cell is determined in part by factors extraneous to its own constitution, it does not necessarily follow that the factors which direct the development of these cells into different avenues will all operate at the same place or at the same time in that truly equipotential system. Neither is the possibility precluded that in some parts this equipotentiality or embryonic condition may persist until a late stage in the ontogeny, making possible a plasticity of interadaptation and a regulation of parts

—a phenomenon long since recognized and known as indeterminate development. The great importance and great complexity of the intercellular environment is little appreciated. We are likely to consider this type of environmental differences as of little importance for the reason that we know little about them.

Again I wish to state as I have twice done previously that "the development of the vascular system furnishes an unproductive field for the solution of the problems of preformation and epigenesis." A diffuse tissue like the mesenchyme is the very worst sort of tissue in which to prove predestination by authority.

Despite the very important work which has recently been done on the vascular system, the interest which attaches to its main problems is by no means merely historic. The vascular system still may have some surprises in store for us. The fact that the problem can be attacked experimentally need by no means belittle its importance. As yet we are still in the stage of testing our methods, and will do well to accept with caution the results of experimental methods, remembering that experimental conditions may obscure the normal process. The very best we can do experimentally is to subject the embryo to as many methods of experiment as possible, selecting results from those methods which alter to the least extent the normal process. By combining the pictures obtained from these various methods into a composite picture we may by chance obtain a resultant which will be of service to us in the study of the normal process. By determining as many as possible of the things which a tissue is able to do, we obtain information as to what it does do. We may, perchance, sometimes find that a given tissue can do some of those things which it had previously been regarded as unable to do; this alone would justify experimental effort.

I wish to make clear that in no case do I regard an experimental condition as portraying a truly normal process, and have therefore not volunteered an estimate of the significance to be attached to the observed conditions in each experimental case. It is too much to dare hope that my results will meet with ready acceptance; all I can say is that during the time in which I have worked, I have spared neither labor nor pains to make these results accurate.

III. SUMMARY

The foregoing results furnish evidence in favor of the following propositions:

First, that mesenchyme in many regions of the body can turn into endothelium, and that endothelium is not an ingrowth from vessels on the yolk.

Second, that prevascular tissue can come from more than one germ-layer.

Third, that mesenchyme cells which can form a given type of blood cell are not confined to a narrowly limited region of the embryo.

Fourth, that endothelium can transform into blood-cells.

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PLATES

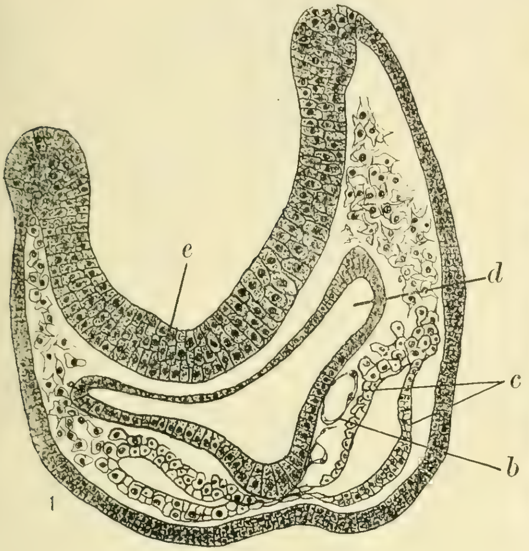
PLATE 1

EXPLANATION OF FIGURES

11 Section through the anterior portion of the forebrain of a head-meroplast, showing unusual head-coelom. Total incubation thirty-two hours. Operation at the time of the first intersomitic groove (× 160). Experiment, Type I, no. 19; *b*, anlage of ventral aorta; *c*, coelom; *d*, pharynx; *e*, forebrain.

12 Section through the forebrain of a head-meroplast, showing early stages in the formation of vasofactive cells. Total incubation, twenty-nine hours: Operation previous to the formation of the first intersomitic groove (× 200). Experiment, Type I, no. 24; *a*, prevascular mesenchyme; *c*, coelom; *d*, pharynx; *e*, forebrain.

13 Section through the forebrain of a head-meroplast showing a loose parenchyma in a position occupied by the isolated vasofactive cells of figure 4. Total incubation thirty hours. Operation at the time of the first intersomitic groove (× 150). Experiment, Type I, no. 18; *a*, prevascular mesenchyme; *c*, coelom; *d*, pharynx; *e*, forebrain.



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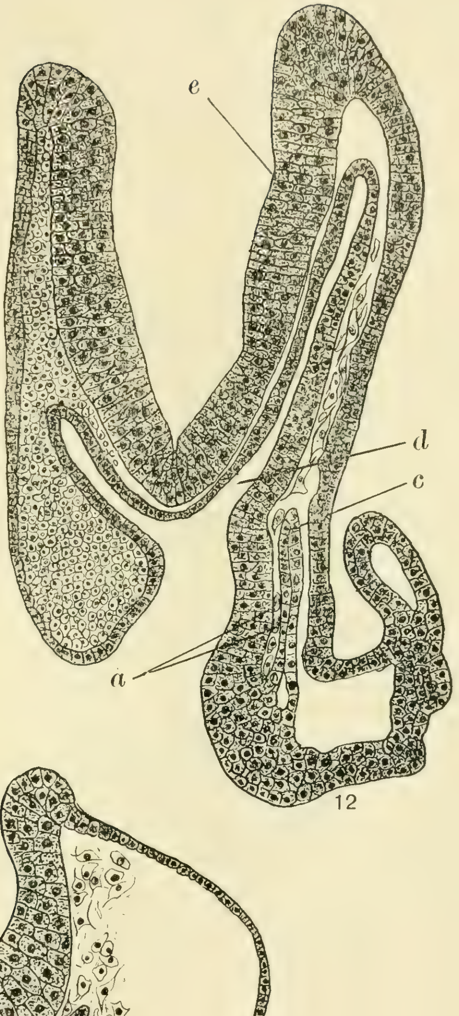

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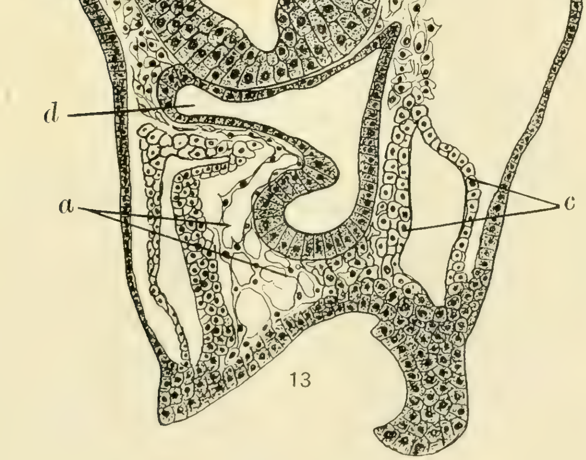

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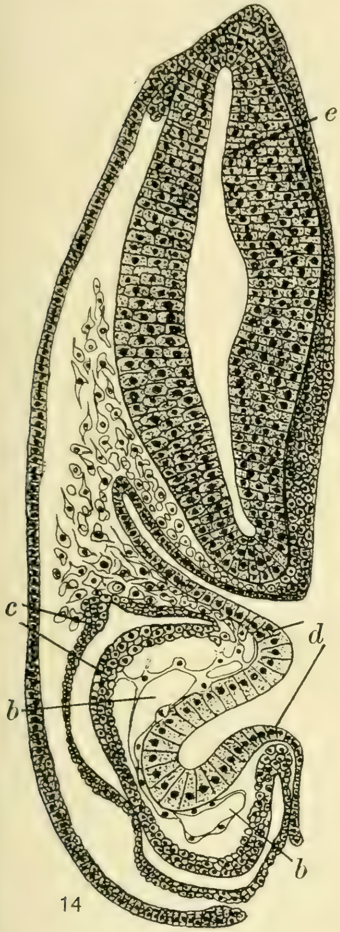
PLATE 2

EXPLANATION OF FIGURES

14 Section through the forebrain of a head-meroplast showing anlagen of the ventral aorta. The longitudinal incision on the right was relatively close to the neural fold. The cut edges of the pharyngeal entoderm have pulled apart the ventral tissues having swung to the left. Experiment, Type I, no. 31 (× 240). *b*, ventral aorta; *c*, coelom; *d*, pharynx; *e*, forebrain.

15 Section through the forebrain of a head-meroplast showing a well-defined ventral aorta. The right longitudinal incision was close to the neural fold. None of the excised head has regenerated; the mesenchyme is very compact near the cut surface the cells of which are somewhat epithelial. Total incubation thirty-three hours. Operation at the time of the first intersomitic groove (× 175). Type I, no. 17; *b*, ventral aorta; *c*, coelom; *d*, pharynx; *e*, forebrain.

16 Section through the forebrain of a head-meroplast showing a proamniotic sac containing a pouch of coelomic mesothelium. On the ventral side of the sac is a peculiar proliferation of entodermal cells very constantly appearing in experiments of this type, generally more symmetrically situated. Total incubation, forty-eight hours. Operation at the time of the second intersomitic groove (× 55). Experiment, Type II, no. 3; *c*, coelom; *e*, forebrain; *f*, ectoderm; *g*, entoderm; *j*, extra-embryonic vessels.



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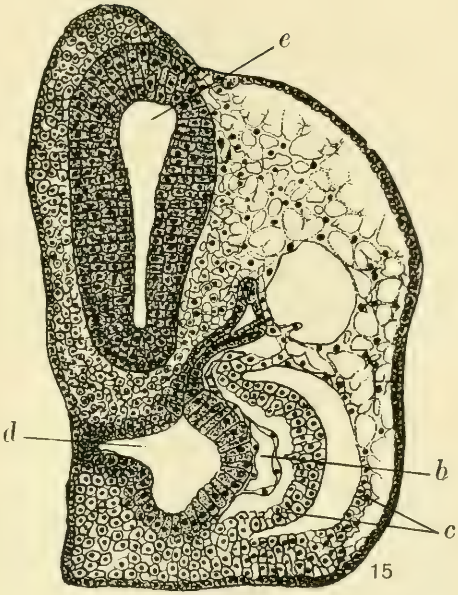

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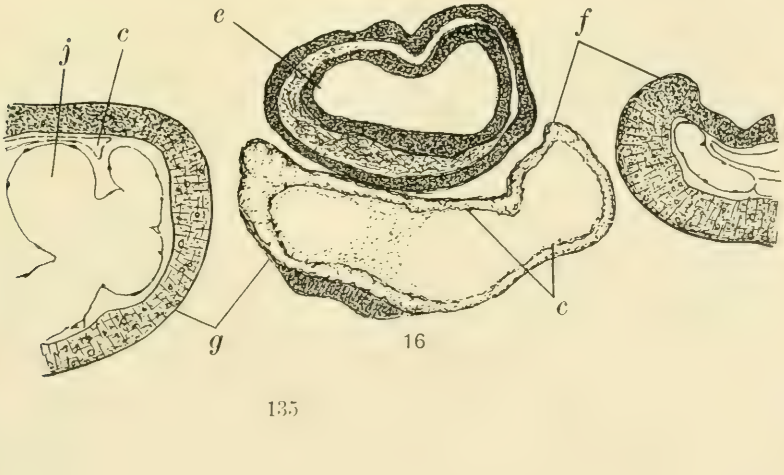

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PLATE 3

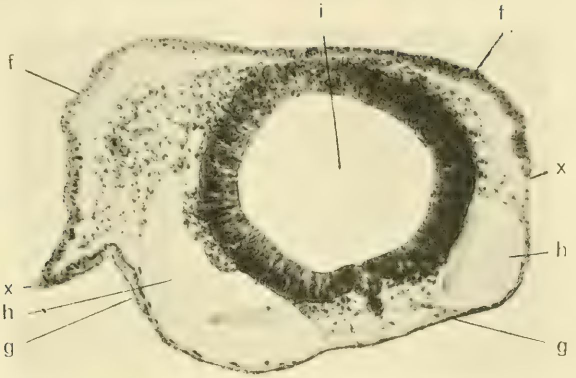
EXPLANATION OF FIGURES

17 Photograph of a section through the midbrain of the same meroplast as in figure 16, showing well developed dorsal aortae and the absence of a tubular pharynx in a tubular head. Fusion of entoderm and ectoderm at points indicated by x (× 120). *f*, ectoderm; *g*, entoderm; *h*, dorsal aorta; *i*, midbrain.

18 Photograph of one of the first available sections of the blastoderm behind the incision *G* to *H* of Experiment Type II, no. 3, showing the freedom of the pellucid area from endothelium (× 160).

19 Photograph of a transverse section of the removed blastoderm of Experiment Type I, no. 31. One of the first available sections behind the transverse incisions, showing especially well the position of the longitudinal incisions and the status of the extraembryonic vascular tissue. *Endth.*, endothelium; *Pv.mch.*, prevascular mesenchyme.

20 Photograph of the removal blastoderm of Experiment Type I, no. 122, showing the absence of all vascular tissue at the time of operation (prior to somite-formation). See also figure 22.



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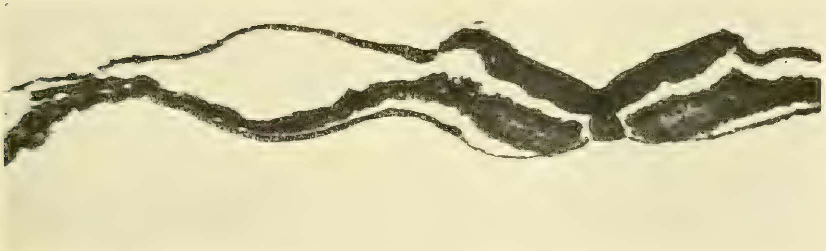

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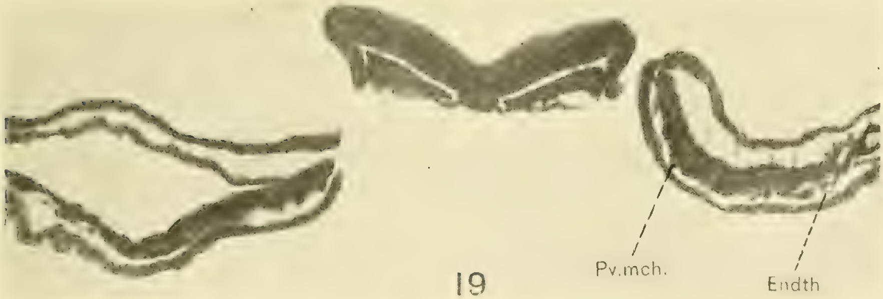

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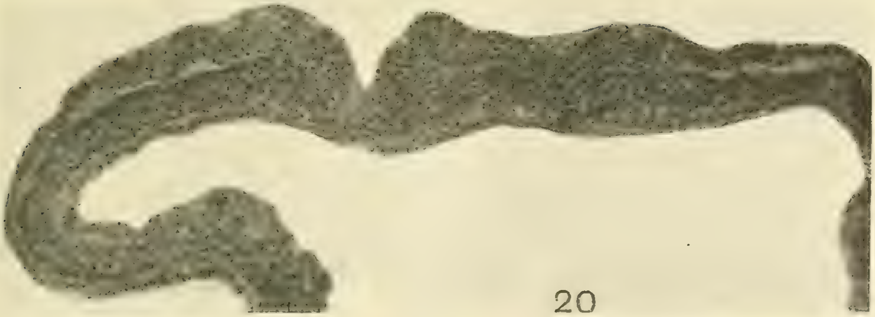

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PLATE 4

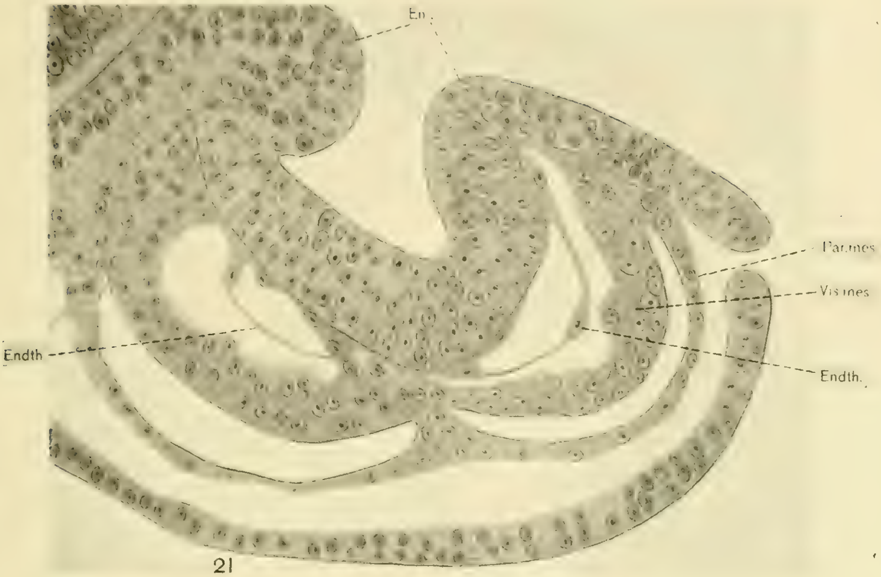
EXPLANATION OF FIGURES

21 Portion of a transverse section through the pharyngeal region of meroplast Type I, no. 31, near the plane of figure 14.

22 Portion of a transverse section through the pharyngeal region of meroplast Type I, no. 122, showing conditions very similar to those in figure 21. The endothelial tubes of the two sides are in communication ventral to the pharynx. The endothelium stains more darkly than does the mesenchyme.

ABBREVIATIONS

Coel., coelom
Endth., endothelium
Ent., entoderm
Par. mes., parietal mesoderm
Vis. mes., visceral mesoderm


Ent.
Par.mes
Vis.mes
Endth
Endth.

21

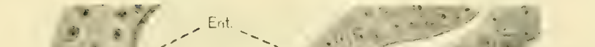

Ent.
Vis.mes.
Endth
Coel
Endth.
Vis.mes

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PLATE 5

EXPLANATION OF FIGURES

23 Section through the posterior heart-region of meroplast Type II, no. 133.

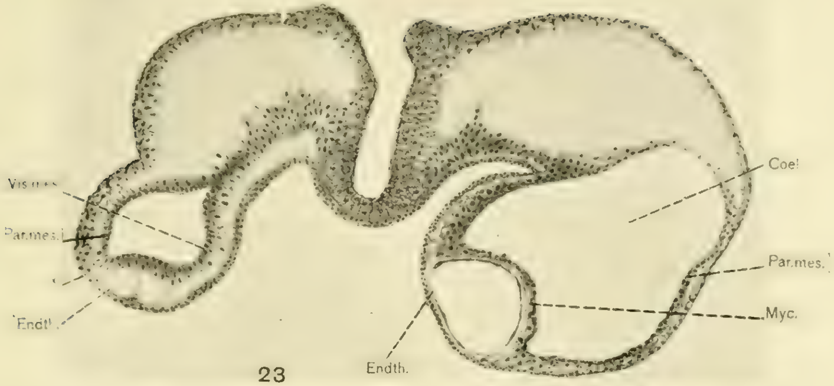
24 Transverse section of meroplast Type II, no. 96. The unilateral heart of this embryo communicates with extraembryonic vessels. The aortic anlagen are discontinuous.

25 Transverse section through the trunk of meroplast Type II, no. 116, showing a beginning of splanchnopleural concrescence.

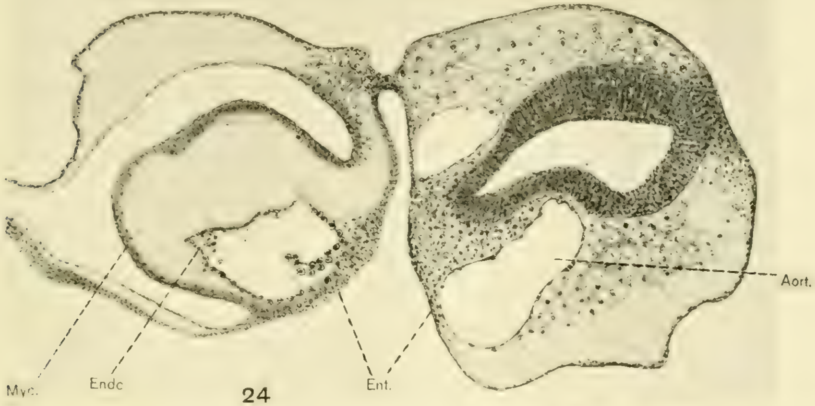
26 Section more anterior in same meroplast showing apposition of concrescing entoderm to close off the pharynx.

ABBREVIATIONS

Aort., aorta
Coel., coelom
Ect., ectoderm
Endc., endocardium
Endth., endothelium
Ent., entoderm
Myc., myocardium
Ot. ep., otic epithelium
Par. mes., parietal mesoderm
Ph. pharynx
Vis. mes., visceral mesoderm


Vis.mes.
Par.mes.
Endth.
Coel
Par.mes.
Myc.
Endth.

23


Aort.
Myc.
Endc.
Ent.

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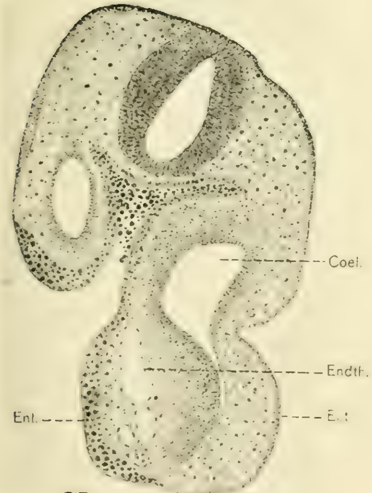
Coel.
Endth.
Ent.
Ect.

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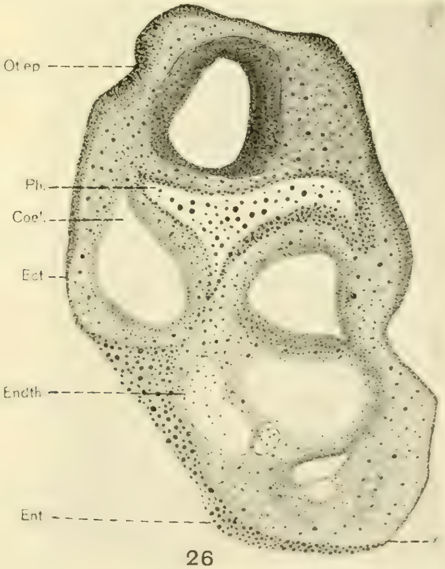

Ot.ep.
Ph.
Coel.
Ect.
Endth.
Ent.

26

PLATE 6

EXPLANATION OF FIGURES

27 Section through the heart of the same meroplast showing completion of ventral 'body wall' and remains of ventral mesentery of the heart. Note the well developed endocardium and dorsal aortae.

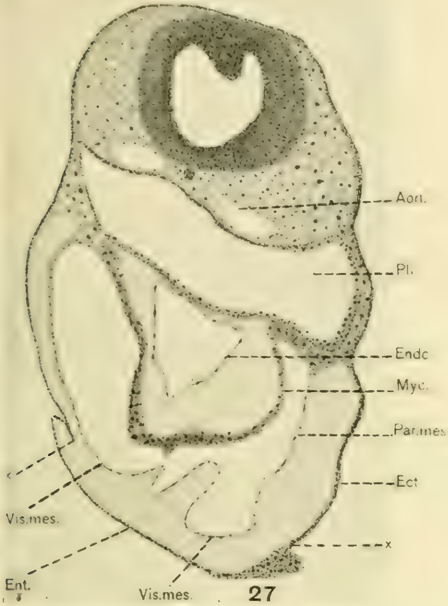
28 Section through the fore-gut of meroplast Type II, no. 52, showing numerous endothelial cysts and mesothelial folds. The entire body wall is covered with ectoderm. Section is slightly frontal.

29 Transverse section through the multiple unilateral heart anlage of meroplast Type I, no. 111.

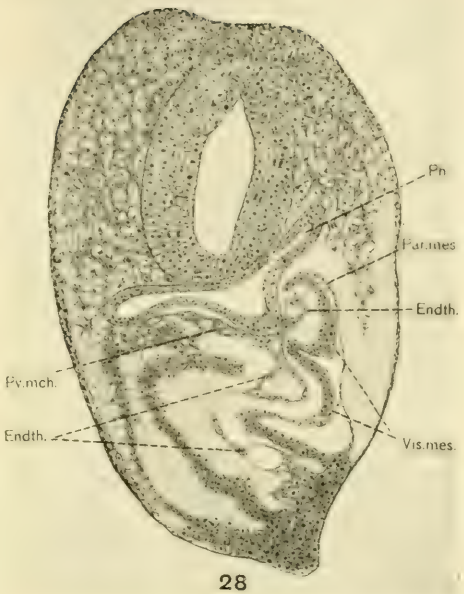
30 Transverse section through the posterior axial region of meroplast. Type II, no. 124, showing unequally developed dorsal aortae.

ABBREVIATIONS

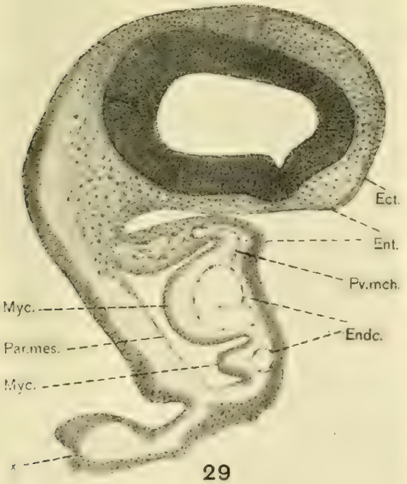
Aort., aorta
Ect., ectoderm
Endc., endocardium
Endth., endothelium
Ent., entoderm
Myc., myocardium
Par. mes., parietal mesoderm
Ph., pharynx
Pv. mch., prevascular mesenchyme
Vis. mes., visceral mesoderm
x, point of fusion of ectoderm and entoderm


Aort.
Pl.
Endc
Myc.
Par.mes
Ect
x
x
Vis.mes.
Ent.
Vis.mes.

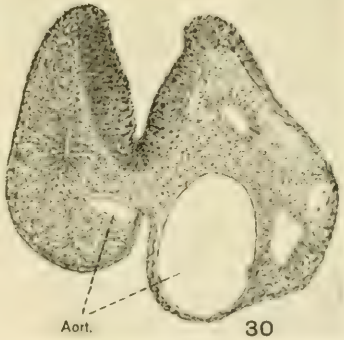
27


Ph
Par.mes
Endth.
Pv.mch.
Endth.
Vis.mes.

28


Ect.
Ent.
Pv.mch.
Myc.
Endc.
Par.mes.
Myc.
x

29


Aort.

30

PLATE 7

EXPLANATION OF FIGURES

31 Section through the posterior axis, Type II, no. 149, showing a small aortic anlage which has developed in a region devoid of a coelom.

32 Section through an early visceral mesodermal proliferation of pre-endothelial tissue in the aortic line anterior to the plane of figure 31.

33 Section between the planes of figures 31 and 32 showing a ventrally directed proliferation of visceral mesoderm in the aortic line.

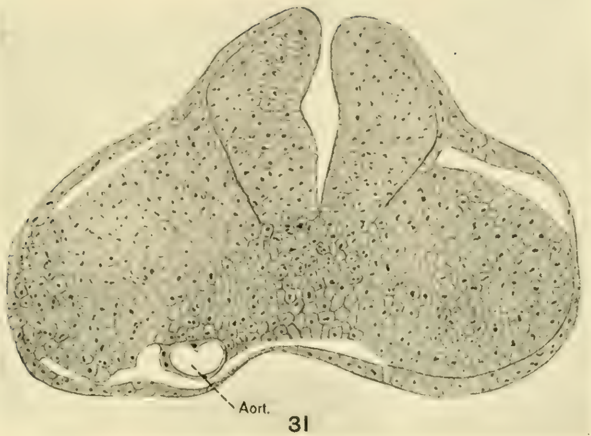
34 Transverse section through the posterior axial region (Type II, no. 53) showing small dorsal aortae.

35 Transverse section through the heart region of Type I, no. 34, showing endocardium approximated to entoderm.

36 Transverse section through anterior heart region of same meroplast showing endothelium apparently being delaminated from mesoderm.

ABBREVIATIONS

Aort., aorta
Endc., endocardium
Ent., entoderm
Pv. mch., prevascular mesenchyme
Vis. mes., visceral mesoderm
x, point of fusion of ectoderm and entoderm



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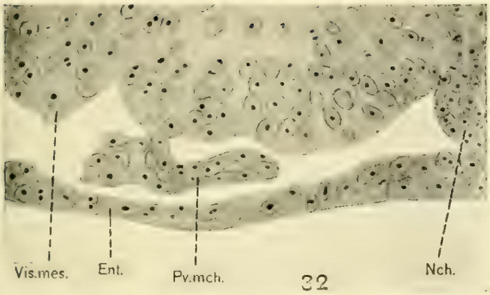

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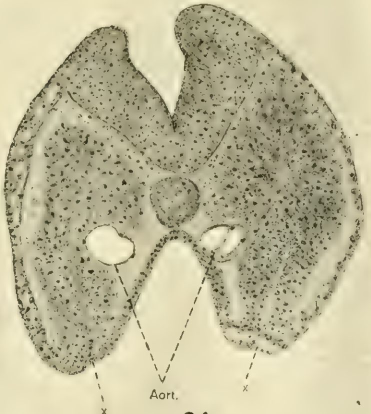

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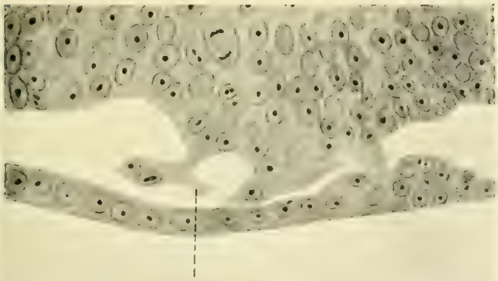

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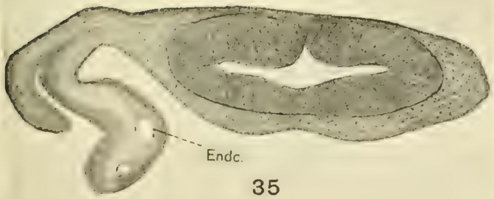

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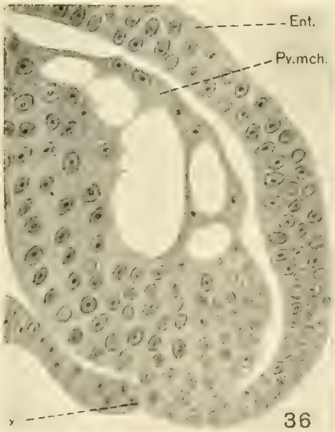

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PLATE 8

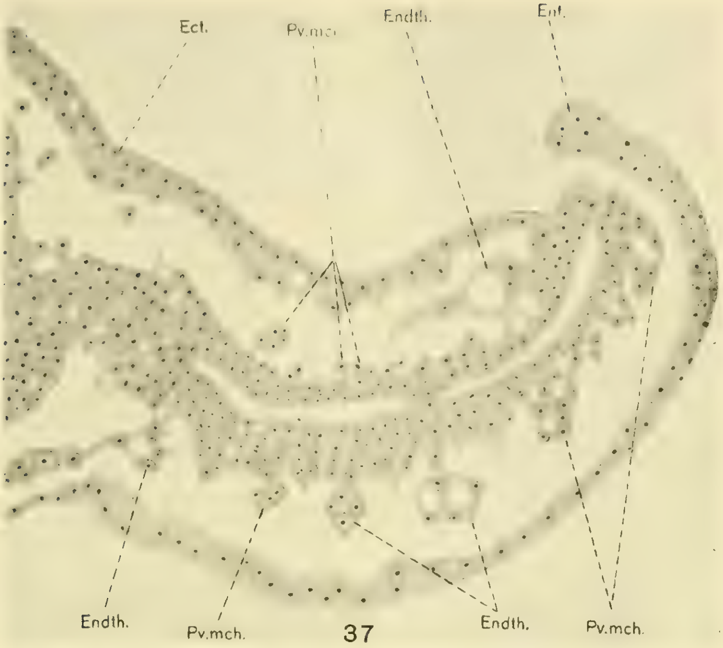
EXPLANATION OF FIGURES

37 Transverse section through trunk of meroplast Type II, no. 88, showing the proliferation of endothelium by visceral and by parietal mesoderm.

38 Section posterior to the plane of figure 37, and more highly magnified.

ABBREVIATIONS

Ect., ectoderm	*Ent.*, entoderm
Endth., endothelium	*Pv. mch.*, prevascular mesenchyme



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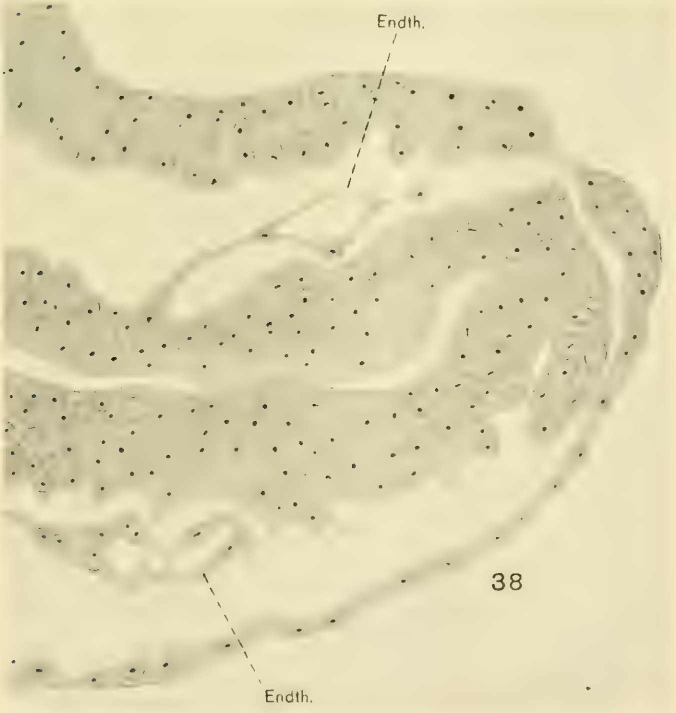

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PLATE 9

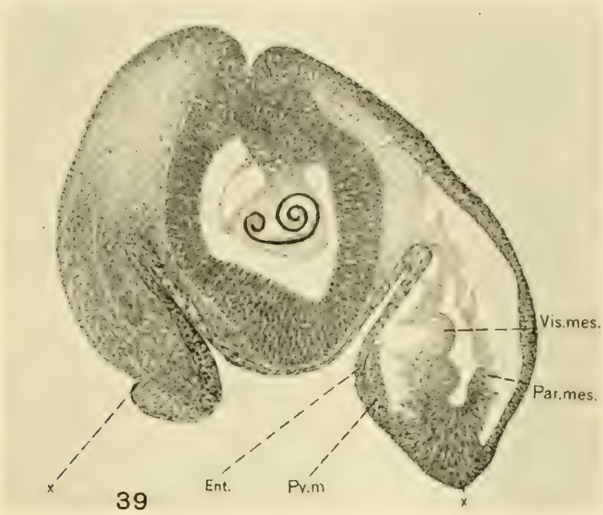
EXPLANATION OF FIGURES

39 Transverse section through the heart-region of meroplast Type II, no. 14. On the right side of the figure will be noted a thickening of the entoderm.

40 Portion of the same section more highly magnified. The mass of cells lying against the entoderm has evidently been proliferated by that tissue.

ABBREVIATIONS

Ent., entoderm
Par. mes., parietal mesoderm
Pv.m., prevascular mesoderm
Vis. mes., visceral mesoderm
x, point of fusion of ectoderm and entoderm



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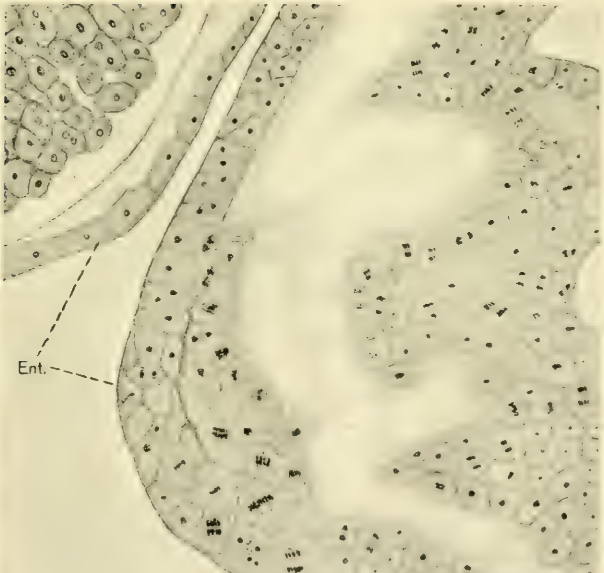

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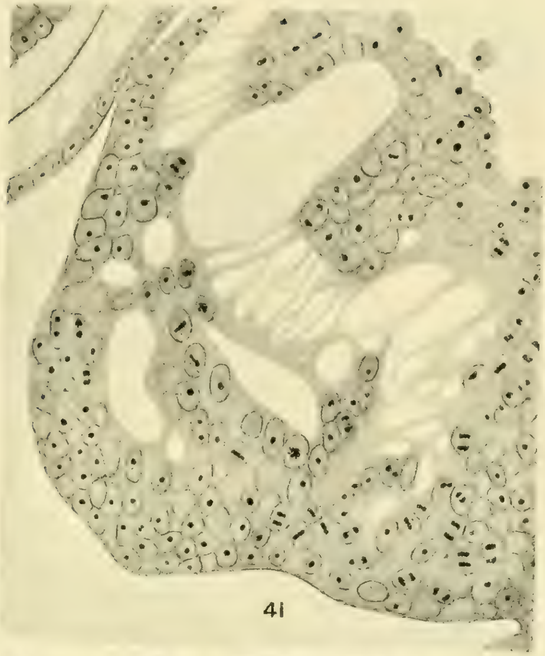
PLATE 10

EXPLANATION OF FIGURES

41 Section slightly anterior to the plane of figure 40, showing the entodermal proliferation slightly separated from the entoderm.

42 Section anterior to the plane of figure 41, one portion of the entodermal proliferation still in contact with the entoderm.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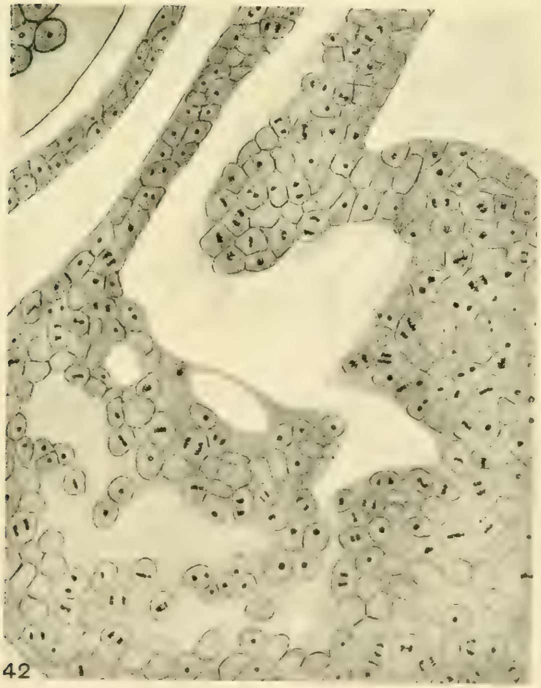
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PLATE 11

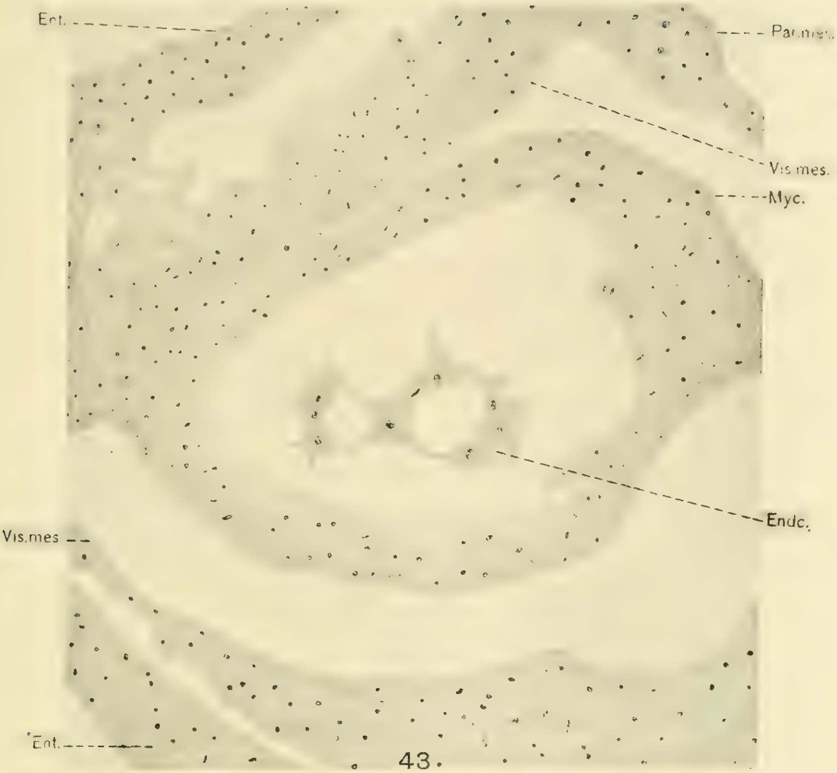
EXPLANATION OF FIGURES

43 Section much anterior to the plane of figure 42, showing a double endothelial tube surrounded by myocardium. This endothelium can be traced back continuously to solid tissue the base of which is shown in figure 40.

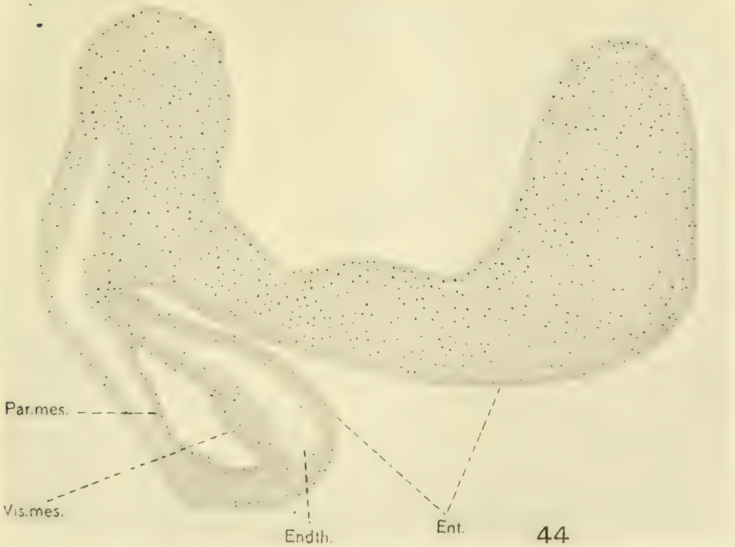
44 Transverse section of the middle heart region of meroplast Type II, no. 48.

ABBREVIATIONS

Endc., endocardium	*Myc.*, myocardium
Endth., endothelium	*Par. mes.*, parietal mesoderm
Ent., entoderm	*Vis. mes.*, visceral mesoderm


Ent.
Par.mes.
Vis.mes.
Myc.
Endc.
Vis.mes
Ent.

43


Par.mes.
Vis.mes.
Endth.
Ent.

44

PLATE 12

EXPLANATION OF FIGURES

45 Section through the anterior heart region of the same meroplast showing an entodermal proliferation of prevascular mesoderm

46 Section between the planes of figures 44 and 45 showing a connection of endothelium with entoderm.

47 Section through the posterior heart region of the same meroplast showing endothelium connected with entoderm.

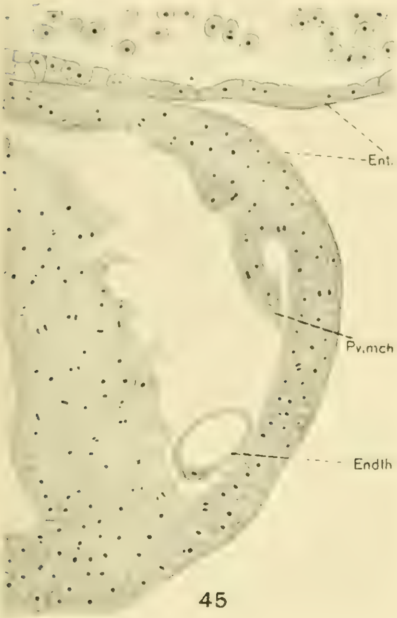
48 Section posterior to the plane of figure showing the prevascular tissue in connection both with entoderm and with mesoderm. The connections with the latter are not mere coagulations of a clear plasma, but are composed of a real intercellular matrix.

ABBREVIATIONS

Endth., endothelium

Ent., entoderm

Pv. mch., prevascular mesenchyme



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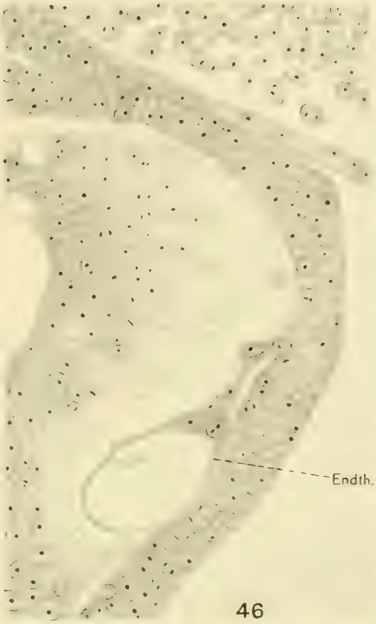

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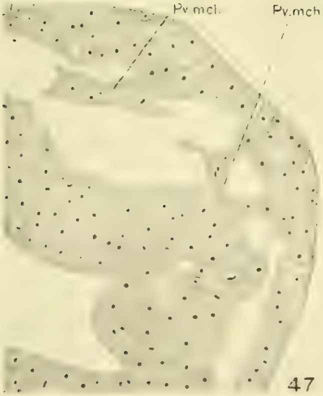

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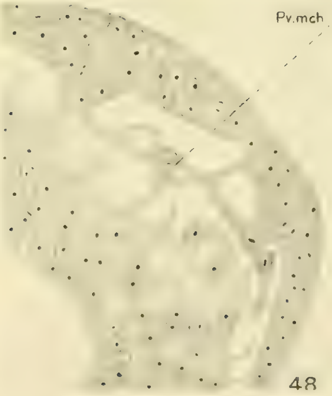

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PLATE 13

EXPLANATION OF FIGURES

49 to 57 Dorsal views of the cephalic portions of chemically treated embryos. Figures 57 and 58 are lateral views. The stippled areas in these figures indicate the location of erythrocytes as observed in the living embryo.

49, 50, 53, 55 Represent the heads of fourteen-day embryos, which at their four-cell stage were treated for twenty-four hours with a solution of 50 cc. sea-water to which had been added 15 cc. of M/12 butyric acid. They are all from the same experiment, one-fourth of the embryos in which yielded similar results. Owing to the evanescence of the red coloring, it is possible that many others may have passed through this critical stage without being observed.

51 and 52 Described with sufficient detail on page 95.

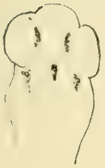
56 and 57 From embryos obtained from the same experiment as that of figures 43 and 44.

58 From an embryo, the treatment of which is described on page 95. *Erth.*, erythrocytes; *Ht.*, heart.

59 Represents an embryo, only a small portion of the body axis of which developed. No heart formation has taken place. There is a large dilated pericardium in the region of which are many isolated erythrocytes. Embryo from same experiment as that from which the embryos of figures 49, 50, 53 and 55 were obtained. It seems improbable that the 'pericardium' of this embryo should be interpreted as Kupffer's vesicle. Even so, there are blood cells of local origin on what would then be the anterior yolk, not shown in this figure.

60 Represents a case in which the embryonic body has failed to develop, or as such has been transformed into mesodermal and vascular tissues which are scattered entirely over the yolk. The embryo at the four-cell stage was treated for ninety-six hours with a solution of 50 cc. of sea-water to which was added 15 cc. of a molecular solution of acetone.

61 A lateral view of a twelve-day embryo treated for twenty-four hours subsequent to the four-cell stage with a solution similar to that employed in case of the embryos represented by figures 49, 50, 53, 55 and 59. Kindness of Dr. E. I. Werber.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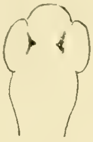
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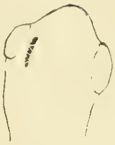
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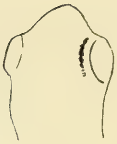
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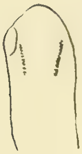
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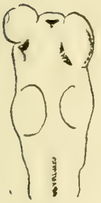
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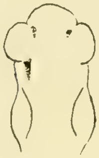
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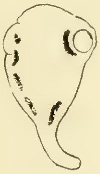
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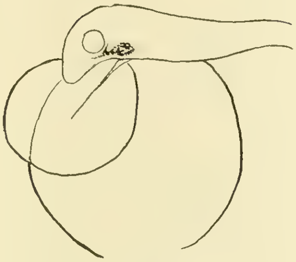
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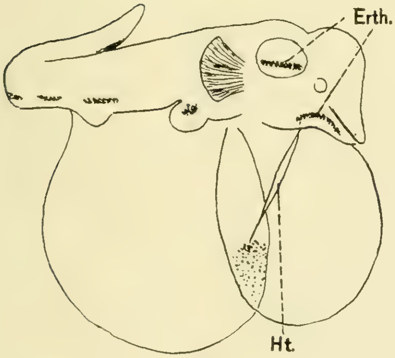

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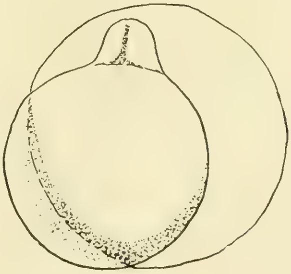
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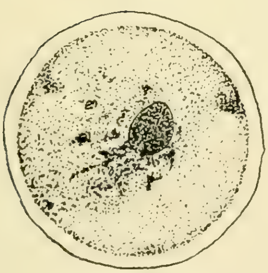
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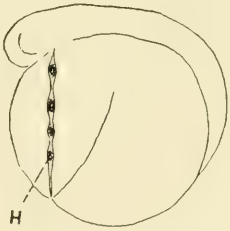


61

PLATE 14

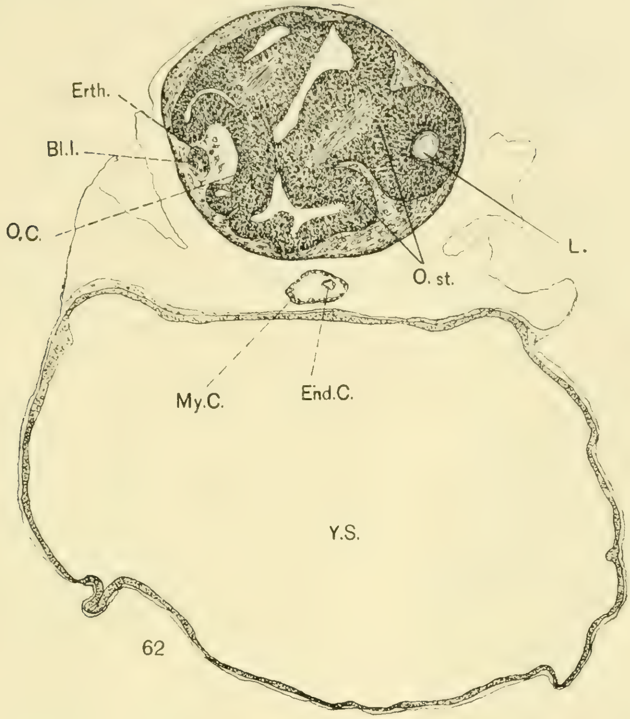
EXPLANATION OF FIGURES

62 A transverse section through the optic region of a nine-day embryo which at the four-cell stage was treated with a solution of 50 cc. sea-water, to which had been added 15 cc. M/12 butyric acid. The yolk-sac is devoid of vascular tissue. The space in the optic cup (on the left side of the figure) normally occupied by a lens, contains a blood lacuna. On the right side is seen the attachment of the upper optic stalk and the base of the lower.

63 Transverse section of the same embryo from which figure 62 is taken; it is a section passing through the optic anlagen, showing isolated crytrocytes, solid heart, and unusual eye-formation.

ABBREVIATIONS

Bl.l., blood lacuna
Endc., endocardium
Erth., erythrocytes
Ht., heart
L., *lens*
Myc., myocardium
Oc., optic stalk
O.st., optic stalk
Y.s., yolk sac



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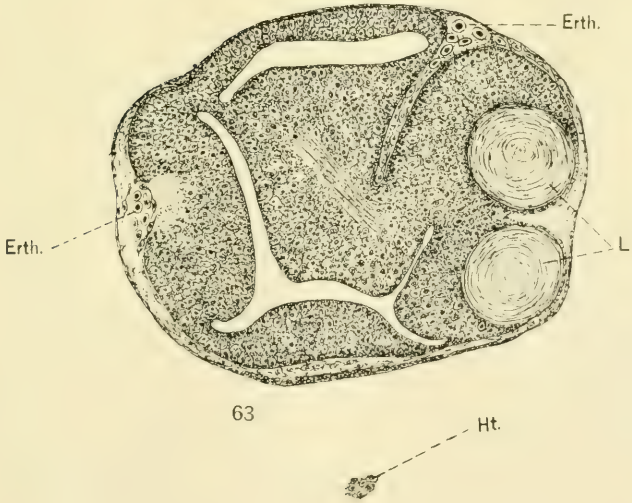


63

PLATE 15

EXPLANATION OF FIGURES

64 Section of the same embryo slightly posterior to the optic stalks, showing the precardinals as haemophoric vessels. The erythrocytes are angular in contour (especially in the vessel on the right of this figure) due to their crowded condition. A few of the erythrocytes in the less crowded vessel on the left have become rounded.

65, 66, 67 and 68 Transverse sections of the embryo from which figure 49 was drawn.

65 Section just posterior to the optic cups passing through the anterior ends of the haematopoetic areas of this region as shown in figure 49. In this figure, as in figure 66, the position of the blood-cells is roughly and arbitrarily indicated by heavy black dots.

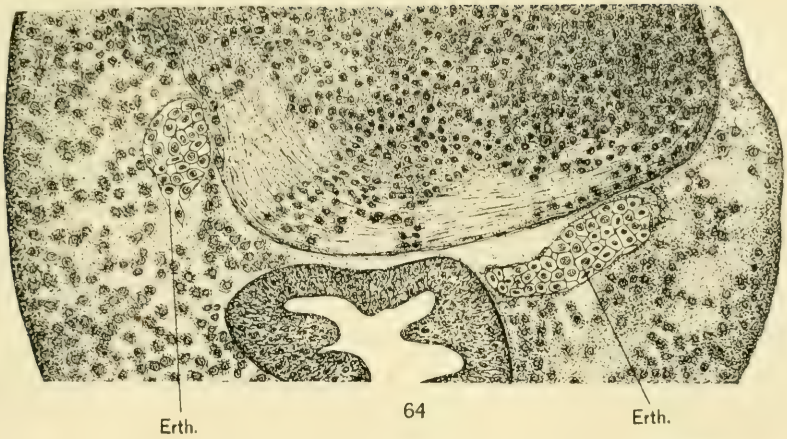
66 Section passing through the posterior portions of the optic cups, and showing the position of the median blood anlage of figure 49. The heart in this figure is a solid continuation of the left portion of the double body labeled heart in figure 65.

67 An accurate detail of the blood-producing areas seen on the right side of the head in figure 65. Conditions in the lower lacuna seem to be farther advanced. Certain mesenchyme cells (lightly stippled) are in a stage of transition to erythrocytes (cytoplasm clear). A blood space is forming while the mesenchyme cells bounding it seem to be receding to form endothelium. The free erythrocytes are rounded in contour.

68 An accurate detail of the median dorsal blood anlage of figure 66. The blood-cells are very strongly eosinophilous but are crowded and possess angular contours.

ABBREVIATIONS

Erth., erythrocytes	*Ht.*, heart



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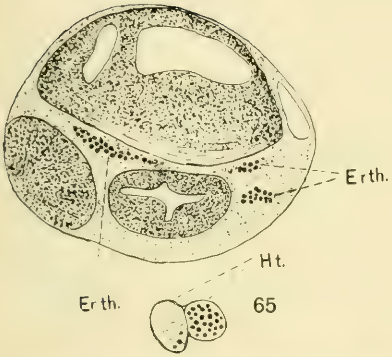

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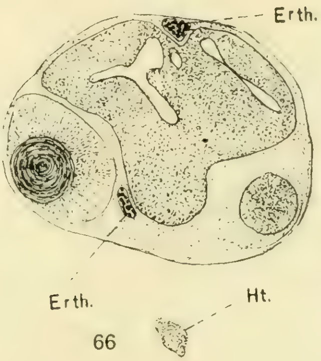

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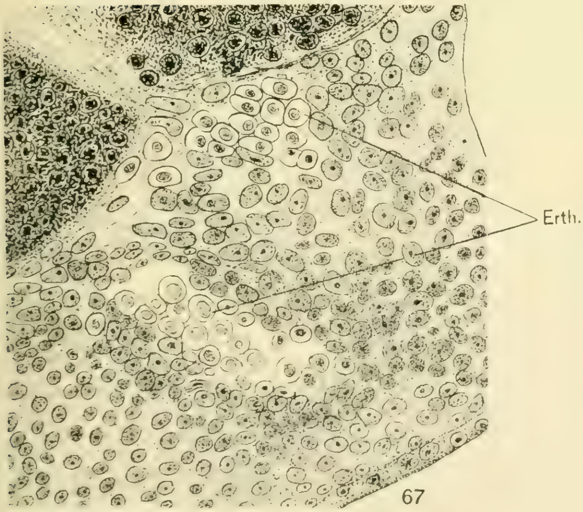

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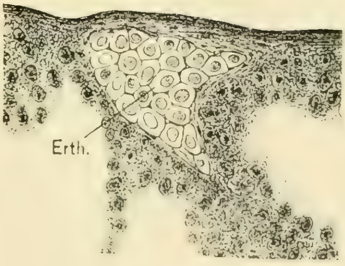


68

PLATE 16

EXPLANATION OF FIGURES

69 and 70 Are described in detail on page 96. The small arrows indicate the direction of pulsation in the 'accessory heart.' Note the apparently normal condition of the actual heart.

71 Section through the heart of this same embryo near the plane of section of figure 65. The connection of the fused portions as described in the text is looser than in the plane of figure 65. The right 'arterial' side contains no endothelium in this section. Erythrocytes in various phases of development are observed. Note the cuboidal nature of the endocardium in the left side of the figure.

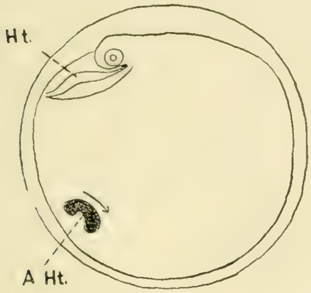
72 Section through the heart of the embryo from which figure 55 is made. In this figure the endocardial cells are cuboidal and rather deeply staining. The endocardial cavity contains erythrocytes which are in a column continuous with the erythrocytes of figures 73 and 74. There is a more or less distinct myocardium.

73 Shows a section of the same heart in which a portion of the endocardium and myocardium are transforming into eosinophilous blood-cells.

74 From the same heart. The plane of section is near the upper end of the heart. The entire endocardium and myocardium of this region has transformed into eosinophilous blood-cells which, as in many instances already noted, are unable to round themselves out for lack of space.

ABBREVIATIONS

A.ht., accessory heart	*Endc.*, endocardium
Bl.l., blood lacuna	*Erth.*, erythrocytes
Myc., myocardium	



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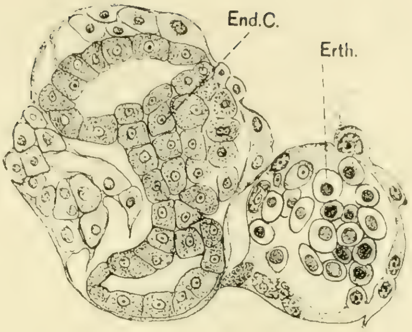

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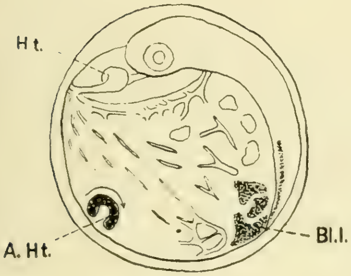

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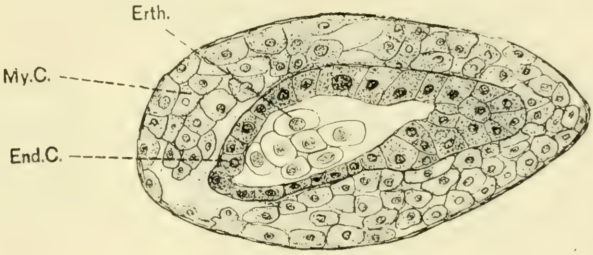

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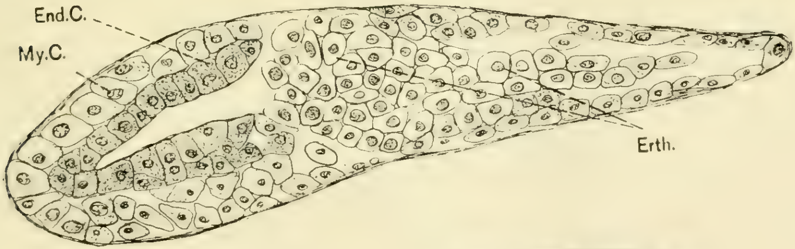

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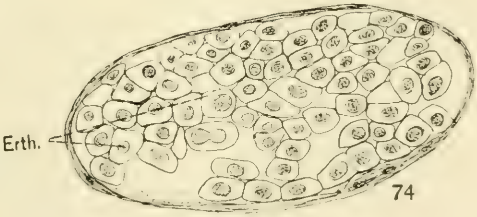

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PLATE 17

EXPLANATION OF FIGURES

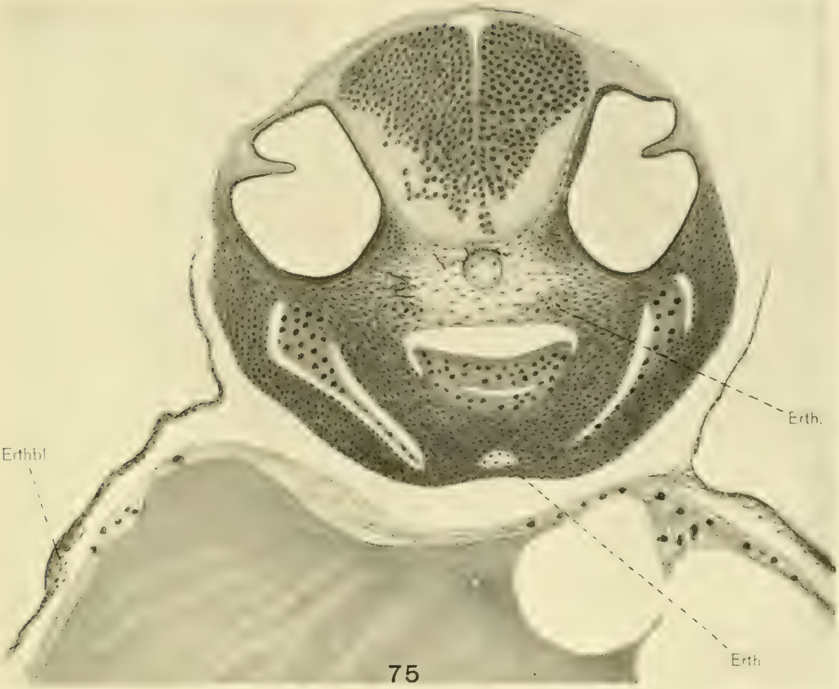
75 Section through the otocysts of the embryo of which figures 62, 63 and 64 are sections. The precardinal lines are indicated by erythrocytes. There are erythrocytes in the very rudimentary short ventral aorta.

76 Section through the optic region of an embryo of Erimyzon sucetta oblongus. Embryo at cell stage was treated with a weak solution of KCN. Dorsal to each eye are erythrocytes in all stages of development.—Kindness of Professor McClure.

ABBREVIATIONS

Erthbl., erythroblast

Erth., erythrocyte



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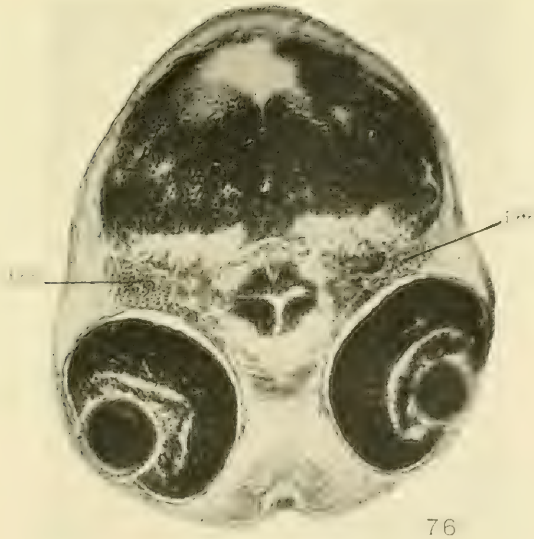

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PLATE 18

EXPLANATION OF FIGURES

77 Section through the heart of the same embryo (see fig. 76) showing the solidity of the proximal portion of the heart and the almost complete absence of anterior vessels.

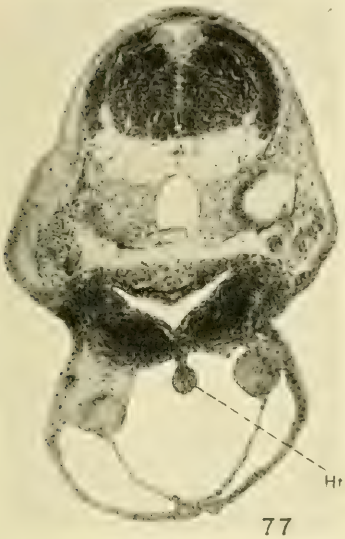
78 Section tangential to the posterior surfaces of the eyes showing the absence of endothelium posterior to the groups of erythrocytes seen in figure 76.

79 Section through the nasal sensory epithelia of the same embryo showing a group of erythrocytes in an endothelial cavity near the sensilla of the left side.

80 Section through the liver of this same embryo showing erythrocytes within the liver tissue and their absence in the aorta.

ABBREVIATIONS

Erth., erythrocytes
Ht., heart
Lvr., liver
Nas. ep., nasal epithelium



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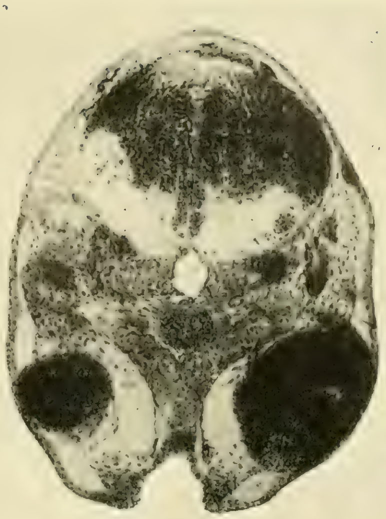

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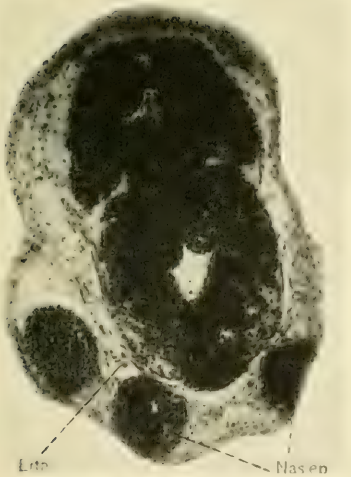

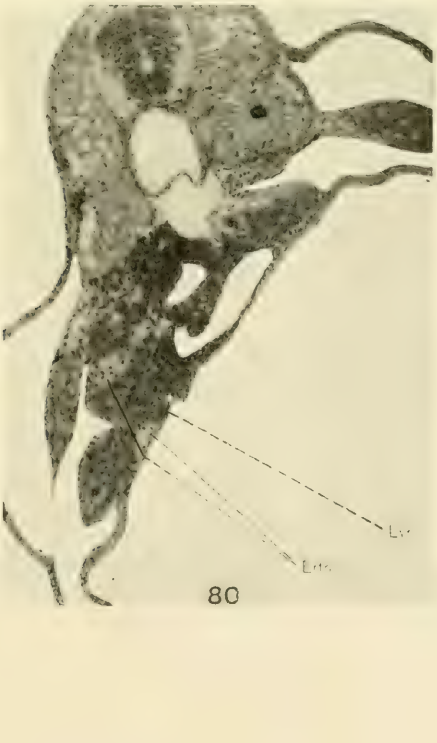

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PLATE 19

EXPLANATION OF FIGURES

81 Section through the liver of the embryo from which figures 62, 63, 64, and 75 are made. Erythrocytes are seen in various stages of formation from mesenchyme cells.

ABBREVIATIONS

Erthbl., erythroblasts	*Erth.*, erythrocytes

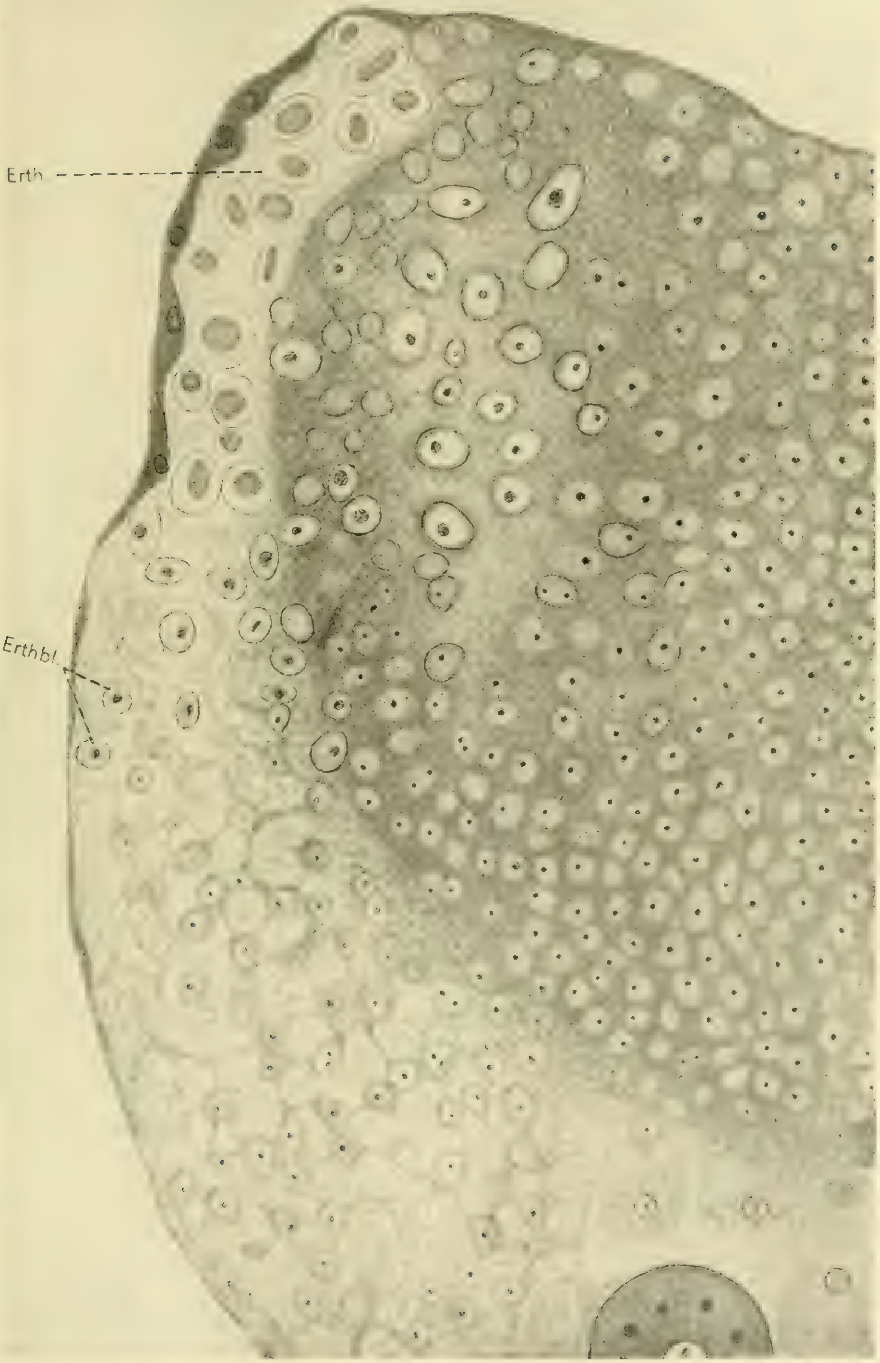
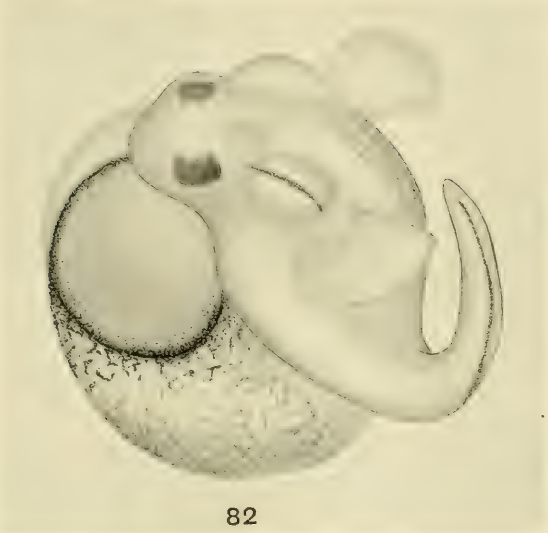

Erth
Erthbl.

PLATE 20

EXPLANATION OF FIGURES

82 Total view of a living cardiectomized embryo of Fundulus. Age nine days. Operation at seventieth hour. Column of erythrocytes can be seen through otocyst. Erythrocytes and endothelium present on anterior yolk but absent on posterior yolk. Eyes very defective.

83 Transverse section through the otocysts of the embryo represented in figure 82. Erythrocytes indicate precardinal lines. *Erth.*, erythrocytes.



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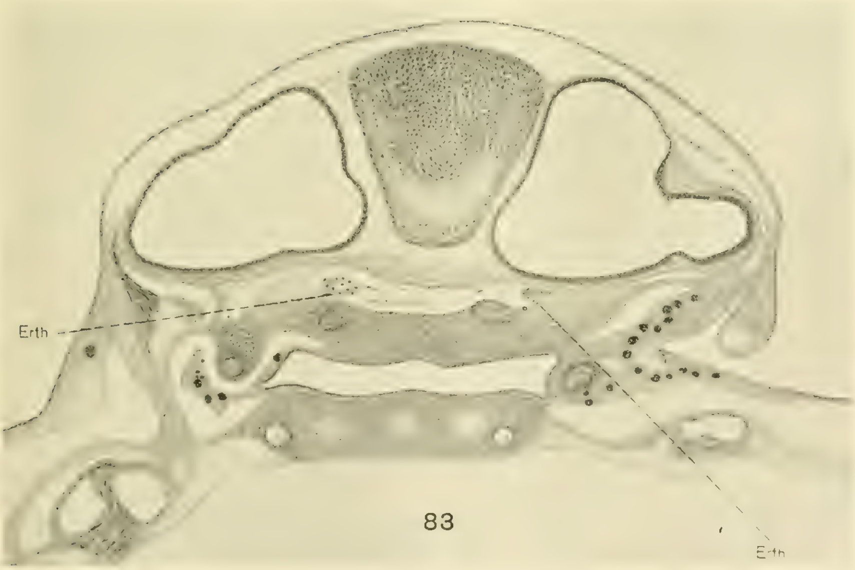


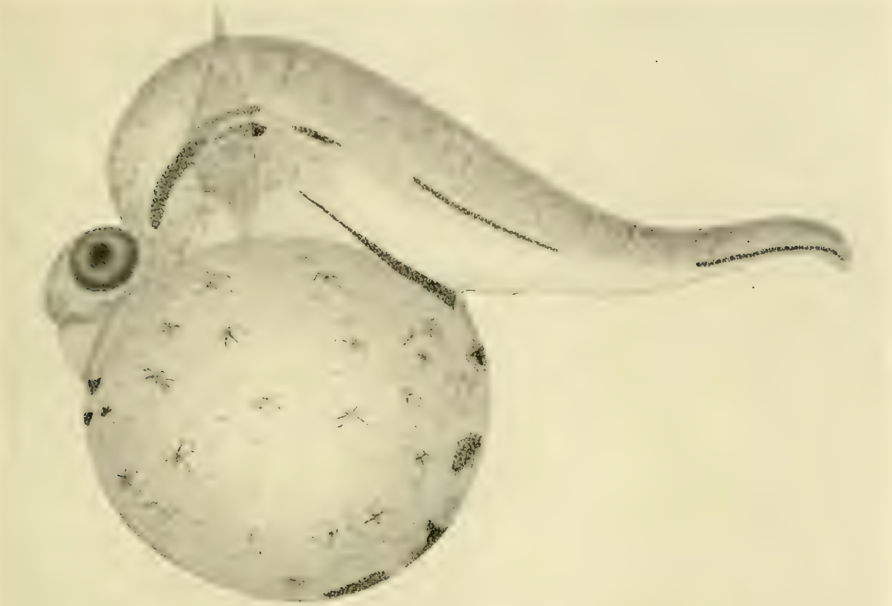
83

PLATE 21

EXPLANATION OF FIGURES

84 A living ten-day Fundulus embryo in which the heart anlage was destroyed at the 60-hour stage. Erythrocytes developed anterior to the pectoral fins, on the anterior yolk just ventral to the pericardium, and in a peculiar position dorsal to the posterior yolk, as well as in the intermediate cell mass and on the posterior yolk.

85 A living twelve-day Fundulus embryo in which the heart anlage was destroyed at the 60-hour stage. At the same time the head was severed. A large 'pericardial' cavity has formed. Erythrocytes formed between the otocysts, in the ducts of Cuvier, and in the intermediate cell mass.



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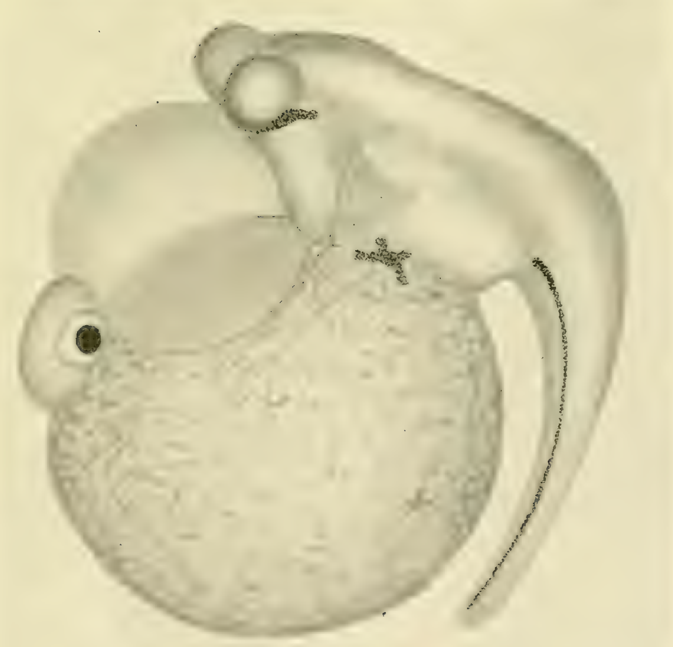


85

PLATE 22

EXPLANATION OF FIGURES

86 Section through the yolk sac of a chemically treated embryo showing the position of a transplanted cephalic meroplast.

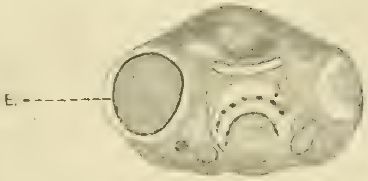
87 The same section of the meroplast more highly magnified showing an otic capsule, and mesenchyme devoid of erythrocytes.

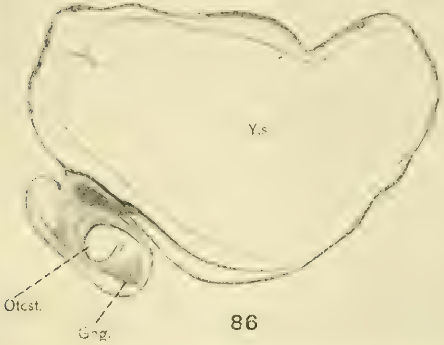
88 A section through the anterior-most region of the meroplast showing the axial portion to be solid cartilage.

89 Section between the planes of figures 87 and 88, more highly magnified showing mesenchyme cells in various stages of transition into erythrocytes.

ABBREVIATIONS

Cart., cartilage
E., eye
Erth., erythrocytes
Erthbl., erythroblasts
Gng., mass of ganglionic tissue
Ot.c., otic capsule
Otcst., otocyst
Y.s., yolk sac





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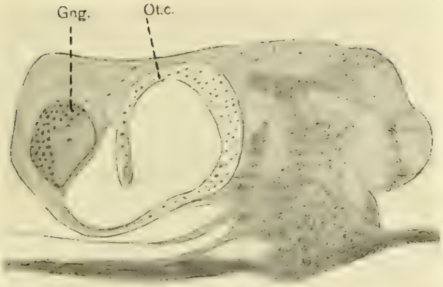

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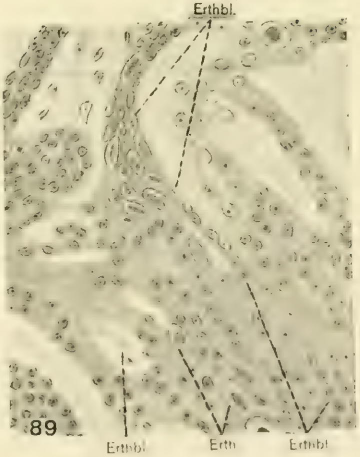

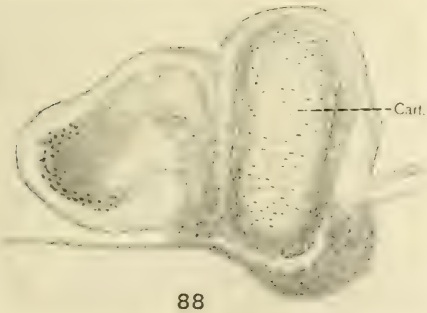

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THE LAWS OF BONE ARCHITECTURE

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TWENTY-EIGHT FIGURES AND FIVE PLATES

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PART I

Introduction

The frequency with which injuries and deformities of the femur are encountered renders the establishment of the laws governing the inner architecture of this bone of great practical and theoretical value. The development of modern views of the significance of the inner architecture of the femur and of bone in general will be briefly considered in a separate section.

The femur has been more carefully and more extensively studied than any other bone. This is easily accounted for by the great complexity of the inner structure, the large size, the unusual shape and the great importance of this bone from a practical point of view. In spite of the large literature and the immense amount of work that has been done upon this subject, there is still much difference of opinion as to the proper interpretation of the inner structure of the femur.

If the mechanical structure of the normal femur is shown to conform closely to the mathematical proportions of a structure of similar shape and physical properties, economically designed to resist the action of loads similar in amount and manner of application, it is conclusive that the structure of the femur is based upon exact mathematical laws: and further, that this is the general law of bone architecture: the form of bone is adapted to its functions.

If the external form and inner architecture of normal bone represent the adaptation to normal function only, then alterations in the static demands made upon the bones must be followed by corresponding changes in both their internal and external structure. Such a transformation of structure would produce deformity if the changed static conditions were sufficiently large to produce a marked change of contour of the normal bone, or to cause a displacement from the natural normal position. Such changes of contour or displacement from the normal must be regarded as a physiological adaptation of the structure to pathological mechanical conditions and therefore to 'pathological function.'

The doctrine of the functional form of bone, with its corallary of the functional pathogenesis of deformity has been ably set forth by Julius Wolff. The foundation upon which this doctrine rests is the correspondence between the inner structure of the upper femur and the lines of stress in the Fairbairn crane, assumed to be of similar shape and loaded in a manner similar to that of the normal femur, the analysis of the crane having been made by the Zurich mathematician, Culmann. The mathematical foundation thus established by analogy alone, has been justly criticised on mathematical grounds so that it must be admitted that the proofs thus far brought forward to demonstrate the soundness of the mathematical basis of this doctrine, though suggestive, are not conclusive.

Being struck with the novelty of the problem of establishing these mathematical relations, and impressed with the great importance of determining the facts by mechanical analysis of the femur by quantitative as well as qualitative methods, in the fall of 1913 I began the studies which have culminated in the preparation of this paper.

Acknowledgments

The anatomical material and supplies required for the prosecution of the studies, the results of which are embodied in this paper, together with the necessary laboratory facilities have been generously provided by the Department of Anatomy, Johns Hopkins Medical School during the past two and a half years.

Grateful acknowledgment is made of the many helpful suggestions received from members of the faculty of this school, and especially from Dr. F. P. Mall, professor of anatomy and Dr. W. H. Lewis, professor of physiological anatomy, both of whom have shown an unfailing interest in this work. Acknowledgment of helpful suggestions is also due Dr. E. R. Clark, professor of anatomy, University of Missouri, formerly of the faculty of Johns Hopkins Medical School, in whose conference course in anatomy I first took up the problem of the mechanics of bone.

It is a pleasure to acknowledge the kindness of my friend, James W. Beardsley, member of the American Society of Civil Engineers, for his suggestions and criticisms of the sections of this paper dealing with theoretical mechanics.

Object of this paper

The object of the paper is the investigation of the normal femur as a mechanical structure: the analysis of its architecture on as strict a mathematical basis as possible: the determination of the quantitative relations that exist between structure and function: the study of the relation of external form and internal architecture: and finally the interpretation of the facts established by my analysis of the femur.

Historical

The earliest mention of the mechanics of bone is accredited to Galileo (1638), who is said by Monroe (1795), to have made reference to the mechanical importance of the form of bone. Galileo had made very important discoveries in the applied mechanics of beams, which even now agree exactly with the most refined methods of analysis.

Duhamel (1743), in a work on anatomy published descriptions of the inner architecture of various bones which were far from accurate. Loder (1805), published an atlas in which were shown pictures of the inner architecture of many bones, including the tibia, femur and humerus. But evidently he understood little of the reasons underlying the arrangement of the cancelli as he describes the head of the femur as consisting of a reticular bony substance.

Not until 1832 was the problem of the mechanics of the inner architecture of bone undertaken. In that year, Bourgery published an anatomy, illustrated by Jacob, in which the inner structure of the femur, tibia, humerus and other bones is delineated with wonderful accuracy. The illustrations are almost as accurate in detail as photographs, but the written descriptions and the interpretation of the inner architecture are very much in error.

Ward ('38), an English anatomist, seems to have been the first writer to grasp, even partially, the real significance of the over-hanging head of the femur and to see any relation between the external form and the arrangement of the cancelli within the bone. His conception of the arrangement of the cancelli in the head of the femur was that they were arranged in straight lines to support the over-hanging load of the femur much as a load is supported by a derrick, where the vertical mast has a cable running from its top to the inclined boom at whose upper end the load is suspended. The stresses in the cable are tensile and in the boom compressive: in like manner, Ward assumed that cancelli run from the axis of the shaft of the femur near the top horizontally, or nearly so, to connect with the sharply inclined lines nearly vertical, that run from the articular surface of the head of the femur to end in the medial part of the shaft.

Jefferies Wyman, an American anatomist, in a paper communicated in 1849, but not published until 1857, advanced the theory of the inner architecture of the femur considerably by analyzing the cancelli of the femur, as seen in frontal section, into three groups: a tensile group, rising from the lateral (outer) portion of the shaft and crossing high in curves to reach about the middle of the head of the femur; a compressive group, rising from the medial portion of the shaft and proceeding radially as straight lines upward to reach either the articular surface of the head, or meeting the lines of the first system at an acute angle and stopping: the third system consisted of numerous short cancelli which bound the two preceding groups together. These observations on the two first groups are correct and in general accord with the facts, but the description and explanation of the position and action of the third group is entirely erroneous.

The earliest German writer on this subject seems to have been Engel ('51), an anatomist of Prague, who made remarkably good observations bringing out some points previously overlooked. He comments on the occurrence in the inner structure of the femur and tibia of the pointed arch, the elliptic arch in various combinations with vertical and inclined buttresses. But the significance of the various geometrical figures he so accurately described was never grasped by him.

In 1858 the English anatomist, Humphry, published his observations on the inner architecture of bone, contributing two very important additions to the knowledge of the inner structure of the head of the femur; that in the frontal section of this bone the lines of cancelli arising from the articular surface of the head are perpendicular to the articular surface at all points; that the two principal groups of cancelli intersect each other at right angles. The importance of these two observations was not recognized for almost a decade, although upon them an important part of the theory of the mathematics of bone architecture depends.

In 1867 Hermann von Meyer demonstrated before a meeting of naturalists held at Zurich a collection of preparations of human bones and discussed the significance of the arrangements of the cancelli in many of the bones. By chance it happened that Culmann, the great Zurich mathematician and engineer, attended the meeting and became much interested in the structure of the bones. He observed that the cancelli of many of the bones were arranged in forms similar to those which he had computed as the lines of maximum internal stress in similar bodies or structures when carrying similar loads. This led to his calculation of the lines of maximum internal stress in a Fairbairn crane having a form which was assumed to approximate that of the upper fourth of the femur. Basing his calculations upon this crane having no hollow spaces he found that the lines of maximum internal stress in this model had similar shapes and positions to the cancelli in the upper femur as shown in frontal section. The conclusion was that these cancelli lie along the paths of maximum internal stress within the bone and thus transmitted a maximum load in the bone with a minimum of material. This discovery of Culmann is the mathematical basis of the modern theory of the functional form of bone.

Von Meyer ('67) published an article describing in great detail the inner structure of various bones together with Culmann's discovery, but incorrectly described the intersections of the cancelli in the head of the femur as obtuse or acute and his illustrations show the cancelli rising from articular surfaces in nearly all cases inaccurately, at an acute or obtuse angle.

Wolff ('69) improved on the technique of his predecessors by using the ivory-worker's saws for making extremely thin sections of bone which were very easily photographed when placed against a dark back-ground. In this manner and with absolute accuracy the detailed structure of the bone could be reproduced and studied. He emphasized the importance of the right angle crossing of the cancelli in the upper femur, which was first observed by Humphry, and called attention to the significance of the analogy between the directions of the cancelli of the upper femur and the tensile and compressive lines of maximum stress, as demonstrated by Culmann's calculations for the Fairbairn crane.

Shortly after appeared a large number of studies and investigations of the subject by numerous writers. It is impossible to review here all the valuable contributions and the extensive controversial literature on the subject. Various writers took up in detail the study of the different bones almost altogether from a morphological point of view, rather than a mathematical or analytical one. Important work was contributed by Zaiijer ('71), Wolfermann ('72), Aeby ('73), Engelmann ('73), Merkel ('74), Langerhans ('74), Bardeleben ('74), Dwight ('75), Bigelow ('75), Rauber ('76), Messerer ('80), von Meyer ('82), Humphry ('88), Lauenstein ('90) and Wolff ('91, '92, '96, '99, '00).

Bardeleben in 1874 published a very careful and detailed study of the vertebral column which is admirably illustrated. Rauber ('76) published his investigation of the elastic properties and the strength of bone as determined by the testing of slabs and prisms cut from various human bones. In 1880 appeared Messerer's elaborate work on the strength of all the important bones of the body as determined by testing to destruction the entire bone. The work of Messerer is in close agreement, however, with Rauber's work, and both constitute a complete demonstration of the mechanical properties of bone as an elastic material.

In 1892 appeared Wolff's classic on the law of bone transformation, in which he developed in final form his theory of the functional form of bone and the transformation of bone in normal and pathological cases, and discussed in considerable detail the structural changes in bone due to changed static conditions. This work is abundantly illustrated with examples of many rare deformities, taken from the principal museum collections of Germany. Culmann's mathematical analysis of the lines of stress in the Fairbairn crane, and the somewhat analogous position of the trabeculae in the upper femur are cited by Wolff as mathematical proofs that the inner architecture of bone follows exact mathematical laws, and that the form and inner structure of bone is determined by the static conditions present in normal and pathological cases. This is supplemented by a detailed discussion of the museum specimens illustrating various deformities.

Wolff's doctrine of the functional form of bone and the functional pathogenesis of deformity have been vigorously assailed on various grounds by many investigators, among whom may be mentioned Zschokke ('92), Lorenz ('93), Ghillini ('98), Schede ('92), Korteweg and Ritter ('88). Zschokke insisted that the inner architecture of bone is designed for resisting only compressive stresses. Ritter presents the novel theory that the curved trabeculae in bone are like the parabolic lines formed by the tiny jets of water which cross each other at random in a water-fall. Bähr ('97) on solely mathematical grounds, without respect to anatomy or clinical studies, denies that the femur acts as a crane at all, and asserts that the Culmann analysis is entirely wrong!

To enumerate all the more or less fanciful theories proposed to account for the form of the inner architecture of bone would be both tedious and confusing. In spite of the general acceptance, at least in part, of Wolff's doctrines, there is still strong opposition to his theories of bone transformation and the functional form of bone, and the soundness of the mathematical basis of these theories has been assailed with numerous mathematical arguments.

The development of Roentgenology has given a new interest to the subject of bone architecture, by permitting comparative studies of the inner architecture to be made upon the living in whom the transformation processes can be readily followed. Such studies made by Sudeck, Gallois and Bosquette ('08), and many other investigators have tended to confirm the soundness of the doctrine advanced by Wolff, although all such studies lack the precision required for the mathematical demonstration of the theories under discussion.

Additional work by Solger, von Recklinghausen ('93), Roux ('80, '93, '96), Graf ('94), Schmidt ('98), Maas ('01), and Fuld ('01) tend to confirm, in a general way, the observations of Wolff. The work of Gebhardt ('01) will be briefly discussed in another section.

PART II. SUMMARY OF MATHEMATICAL PRINCIPLES

Introduction

A fundamental idea of mechanics is necessary to an understanding of the application of mechanics to the problem presented in the analysis of the femur. It will be manifestly impracticable to explain these principles of mechanics, which are applied to this problem, in great detail and only the essentials that are universally accepted by mathematicians, engineers and scientists will be given.

A clear understanding of the definitions and mechanical principles given in the following paragraphs, *1* to *53* inclusive, is indispensable in solving problems involving forces and stresses. Obviously, in a paper of this nature a discussion of mechanical principles must be brief and confined to the essentials. The interested reader can find a full discussion of the subject in any of the standard text-books of mechanics.

Definitions

1. Mechanics is that branch of dynamics that deals with the laws that govern the action of forces upon solid bodies.

2. Statics is that branch of mechanics that deals with the laws and conditions of forces acting upon solid bodies at rest, as for example, bridges, buildings, arches and other structures.

3. Graphic statics deals with the graphic representation of forces acting upon solid bodies at rest.

4. Force is that action of one body upon another which tends to change, or changes, the state of rest (or motion) of the body acted upon. Force has direction, magnitude, and a point or place of application, and is defined when these are known. The line of action of a force is the line drawn through the point of application of the force, or through the point at which the force may be regarded as concentrated, parallel to the direction of the force. The usual unit in use as a measure of force is the pound (or ton, kilogram, etc.), which is the force exerted by gravity upon the standard weight also called a pound (or ton, kilogram, etc.).

5. Effect of force. A force is the cause that may produce the following effects: a) an opposition or balancing of other known forces; b) change of motion of the body either in direction or velocity; c) a measurable distortion of the solid body itself. The second effect relates to the study of the laws of motion and need not be further considered; the first and third apply to the subject of statics.

6. Stress is the internal force which, when a body is acted upon by external forces, resists the change of position of the molecules of the body and tends to preserve their original position. Stress, like force, is measured in pounds (or tons, kilograms).

7. Deformation is the change of shape which is produced by the action of equal but opposite forces and is measured in units of length—inches (feet or millimeters). The units, inch and pound, will be used throughout the following discussion.

8. Unit-stress is the stress per square inch of cross section =

$$\frac{\text{total stress}}{\text{total area of cross section}} = \frac{P}{A}$$

Unit-deformation is the ratio $\dfrac{\text{alteration in length}}{\text{original length}} = \dfrac{d}{e}$

9. Compression (fig. 1, *a*, *b*, *c*) is the stress which tends to keep adjoining planes of a body from being pushed together by the action of two equal and opposite forces acting toward each other. If a vertical post of uniform cross section is supporting a load of 1000 pounds uniformly distributed over the surface of the top, for equilibrium there must be an internal force or compression in the post, equal to this load, or a compression of 1000 pounds.

10. Tension (fig. 1, *d*) is the stress which tends to keep adjoining planes of a body from being pulled apart by the action of two equal and opposite forces acting away from each other. If 1000 pounds is suspended from a rigid support by a steel bar of uniform cross section there will be in every section of the bar an internal stress produced by this load: for equilibrium this stress or tension must be equal to the external load—1000 pounds.

11. Shear is the term applied to the stress (internal force) which tends to keep two adjoining planes of a body from sliding one past the other, under the action of two equal and parallel forces acting in opposite directions. These forces that produce shear are called shearing forces and are usually very close together so that their action is similar to that produced by a pair of shears, hence the name. As an example of shear, in figure 1 *c* let B represent a block of an homogeneous material rigidly fixed in a vertical plane and resting between blocks A and C, as shown, with the left edge of A and the right edge of C in the same vertical plane. A vertical force, P, applied to B through the block A will be transmitted to block C by means of B and will develop in B a shear equal to the force transmitted. Shearing force is usually assumed to be uniformly distributed over the area of the cross-section upon which it acts.

If a straight bar of homogeneous material be rigidly fastened at one and at the other a twisting force be applied, the condition of stress produced is called torsion. This is a special type of shearing stress.

12. Elasticity is the property possessed by a material of returning to its original dimensions and shape when the external forces producing distortion are removed.

13. Elastic limit. Every solid body is elastic within certain limits, and the deformation produced in it is directly proportional to the force producing it and to the internal force resisting it. If the force producing deformation exceeds a certain limit the distortions will increase more rapidly than the forces; and when the load is removed the body will not entirely regain its former shape and dimensions, but will have a permanent set. The maximum unit stress up to which the deformation increases proportionally to the stress is called the elastic limit. This limit varies with the kind of material and the kind of stress, tension, compression and shear.

14. Ultimate strength is the maximum unit-stress which is reached previous to rupture when a body is tested to destruction (as in a testing machine).

15. The modulus of elasticity is the ratio $\frac{\text{unit stress}}{\text{unit deformation}}$, and varies with the material and the kind of stress to which it is subjected. Generally the modulus of elasticity used is that for tensile stress, and is represented by the letter E.

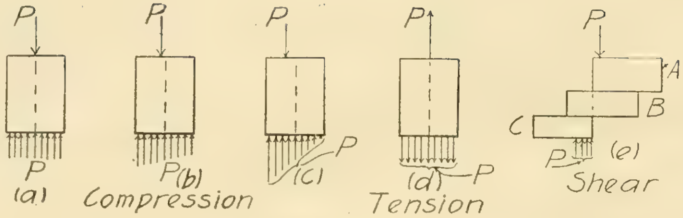


Fig. 1

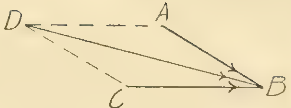


Fig 2

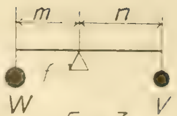


Fig 3

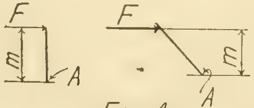


Fig 4

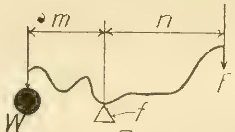
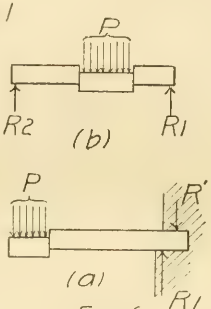


Fig. 5 (a)



(b)

(a)

Fig. 6

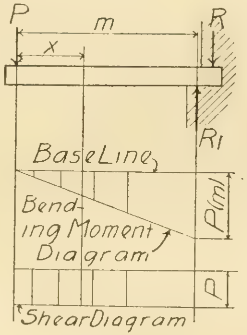


Fig 7 (a)

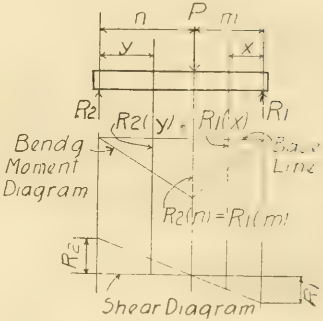


Fig 7 (b)

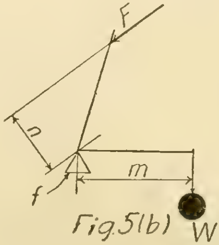


Fig. 5 (b)

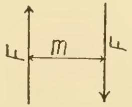
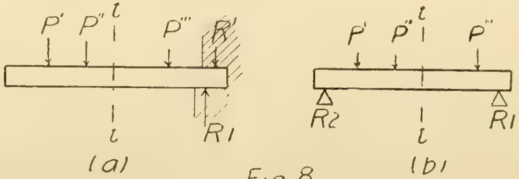


Fig 5 (c)



(a)

(b)

Fig 8

If P is the total tensile stress in pounds,
A is the area of cross section in square inches,
l is the original length of bar in inches,
d is the total deformation in inches.

then

$$E = \frac{\text{unit stress}}{\text{unit deformation}} = \frac{\frac{P}{A}}{\frac{d}{l}} = \frac{Pl}{Ad}$$

In these units E is expressed in pounds per square inch. The modulus of elasticity is constant with very few exceptions for any given material for stress below its elastic limit, but after passing the elastic limit, it steadily decreases. Working unit

Fig. 1 *a* illustrates the action of a compressive force applied in the axis of a given body: Fig. 1 *b*, the action of a compressive force applied at a slight distance from the axial line and parallel to it: Fig. 1 *c*, the action of a compressive force applied parallel to the axis of the body but at a distance of one-sixth of the width of the body from the axis, which produces a pressure in the base of the assumed prism varying uniformly from zero on the edge farthest from the line of action of the force to exactly twice the average pressure, at the opposite edge. Fig. 1 *d* shows the action of a tensile force on a body and figure 1 *e* illustrates shearing action in a given body. Paragraphs *9, 10, 11.*

Fig. 2 Parallelogram of forces constructed for the graphic determination of the resultant of two forces acting at the same point, the direction and magnitude of both being known. Paragraph *17.*

Fig. 3 Action of levers, explained in paragraph *18.*

Fig. 4 Principle of moments explained in paragraph *18.* Figs. 5 *a* and *b* Principle of levers applied to levers of any shape, explained in paragraph *20.*

Fig. 5 *c* Action of a couple, explained in paragraph *21.*

Fig. 6 *a* Action of vertical shear in a cantilever beam. Paragraphs *24, 25.*

Fig. 6 *b* Action of vertical shear in a simple beam resting on two supports.

Figs. 7 *a* and *b* A method of constructing graphic diagrams of the vertical shear and of the bending moment for every part of a cantilever, and of a simple beam, respectively, carrying a single concentrated load, *P*. Complete discussion in paragraphs *26–32.*

Figs. 8 *a* and *b* Indicate the position of vertical loads on a cantilever beam and on a simple beam and show a plane passed through the beams in the line *t–t*. Explained in paragraphs *29–33.*

stresses, or stresses which the body may carry constantly or repeatedly without damage, should always be well below this limit. If the modulus of elasticity is known for a given material, it is easy to compute the deflections or deformations of beams or other structures when the forces acting upon them are given. Conversely, by measuring the deformations due to external loads the stresses in various parts of a structure may be determined.

Composition and resolution of forces

16. The resultant of two or more forces acting in the same plane is that single force which will produce the same effect as the combined original forces.

17. The components of a force are those forces which by their combined action will produce the same effect as the original force.

In figure 2 let AB and BC represent both direction and magnitude of two forces acting through the point B. Through A and C if lines are drawn parallel respectively to BC and AB, intersecting in the point D, the diagonal DB represents the direction and magnitude of the resultant of the two forces AB and BC. To balance AB and BC a third force acting at B and equal to DB but acting in the opposite direction would produce equilibrium.

18. Levers and moments. The simplest illustration of levers is the see-saw. In figure 3 is shown a simple lever supported by the fulcrum f. For equilibrium it is evident that if the weights W and V are equal, their distances from the fulcrum must also be equal. Also, if the weight W is greater than the weight V, the lever arm of W must be less than that of V; and this relation must be such that W times its lever arm, m, or distance from the point of support is equal to V times its lever arm, n. That is, for equilibrium, $W \cdot m = V \cdot n$

These products Wm and Vn are called moments and f is the center of moments. This conception of the lever and of moments is one of the most important principles in mechanics. A force acting on a body tending to rotate it about a certain point is said to produce a moment about that point, equal to the magnitude of the force (in pounds) multiplied by the perpendicular distance (in inches) from the line of action of the force to the point considered. The point about which the force tends to cause rotation is called the center of moments.

A general application of moments is shown in figure 4. The force F acts at a distance m, from the center of moments, A. The distance m, is called the lever arm. The moment of the force F about the point A is equal to F times m, the result being expressed in inch-pounds according to the units of force and length herein used.

19. There may be any number of forces tending to rotate a body about a given point, either in the same or in the opposite direction. If the body is in equilibrium the total moment in a clock-wise (+) direction must be equal to the total anti-clockwise (−) moment, otherwise rotation would occur.

20. A lever may have any shape. Regardless of the actual length of the lever, the true lever arm is the perpendicular distance from the center of moments to the line of action of the force. Figures 5 *a* and *b* illustrate this principle.

21. *A couple,* in mechanics, is a system of two parallel and equal forces acting in opposite directions. If each of these forces is represented by F, and the perpendicular distance between them is m, the moment of the couple is $F \cdot m$. A couple tends to revolve the body upon which it acts and equilibrium can be established only by a couple producing the same moment in the opposite direction. Figure 5 *c* illustrates the action of a couple.

Theory of beams

22. *Reactions.* Bending stress occurs in a bar resting in a horizontal position upon one or more supports. The loads on the bar and its own weight cause it to bend and produce in it complex stresses and elastic deformations which may be resolved into stresses of tension, compression and shear.

Throughout this discussion of beams, paragraphs *22* to *42* inclusive, it is assumed that the cross section of the beam is uniform throughout its length.

23. If a beam rests upon fixed supports which sustain it and its loads, the forces acting through the supports and resisting these weights are called reactions. The reactions upward balance the loads downward. If the position and amount of the loads carried by a beam are known it is easy to compute the amounts of the reactions from the principles of equilibrium already considered. For equilibrium we must have:

Algebraic sum of all forces $= 0$
Algebraic sum of all moments $= 0$

24. Vertical shear. A beam may fail by shearing in a vertical section as shown in figure 6 *a* for a cantilever beam, and in figure 6 *b* for a beam on two supports. This shearing is caused by two equal, parallel forces acting in opposite directions very near the same section.

25. The vertical shear varies considerably at different sections. It can be readily seen that the greatest tendency to shear is at the supports where the loads are transferred from the beam to the supports. Near the supports the vertical shear is equal to the reactions. By simple additions and the application of the laws of equilibrium the vertical shear at every part of a beam may be readily determined.

26. Beams. Beams usually fail by cross-breaking or rupture transversely. Throughout Part II for simplicity, the weight of the beam itself will not be considered in the analysis. In figures 7 *a* and 7 *b* the following notation is used:

P = any concentrated load.
m = distance from right support to line of action of P.
R_1 = reaction of cantilever, figure 7 *a*.
R_1, R_2 = reactions of simple beam, figure 7 *b*.
n = distance from left support to line of action of P, in figure 7 *b*.

Let figure 7 *a* represent a cantilever beam carrying a single concentrated load P, applied at any point along the beam at the distance m from the support. The tendency of the load P to cause rotation about the point of support of the beam is measured by the bending moment produced at that section.

If the load P is at the distance m from the support, the moment about the support is P times m, which is the bending moment at the support. At any point at the distance x from P, between the load P and the support, the bending moment is equal to P times x. These products vary uniformly from zero, at the point of application of P, to a maximum $P \cdot m$ at the support. The amount of the bending moment at any point will be shown graphically, if the product $P \cdot m$ is measured to suitable scale vertically downward under the point of support, and from a horizontal base line; then from the point on the base line under the point of application of the force P, a line is drawn to the ordinate erected under the support. The triangular figure, figure 7 *a*, thus drawn is the bending moment diagram. Scaling the vertical ordinate between the base line and the last drawn line will give the amount of the bending moment for the section of the beam vertically above the ordinate.

In figure 7, *b* is shown the bending moment diagram for a simple beam on two supports, carrying the load P.

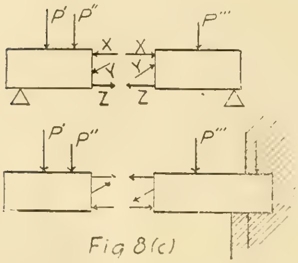
27. The vertical shear for the single load P on the cantilever remains the same for every section of the beam from the point of application of this load to the support. It is represented graphically to scale by drawing a line parallel to the horizontal base line, at a distance measured to any convenient scale, as one inch = 100 pounds shear. The diagram giving the values of the vertical shear at all points in the beam is called the shear diagram. Such a diagram is drawn for the cantilever and is shown in figure 7 *a*.

Figure 7 *b* shows a shear diagram constructed for the simple beam resting on two supports.

28. Relation between internal stresses and external loads. All external forces acting on a beam maintain equilibrium by means of internal stresses produced in the beam by these forces. Since in any section of a beam the external forces produce bending moment and shear, the problem is to determine the relation between these and the internal stresses in any given section.

29. Take any beam loaded in any manner and imagine a vertical plane cutting the beam at any section *t–t* as in figure 8, *a*, *b*. At this section there are in action unknown stresses of various directions and amounts. Suppose the beam separated into two parts by this plane, and let the forces *X*, *Y* and *Z*, equivalent to the internal stresses, be applied as shown in figure 8, *c*. Then the equilibrium of each part will be unaffected, for each part will be acted upon by a system of forces that are in equilibrium.

30. From this we may deduce the fundamental principle that "The internal stresses in any cross section of a beam hold in equilibrium the external forces on each side of that section." This applies to all beams of whatever cross section or nature of loading.



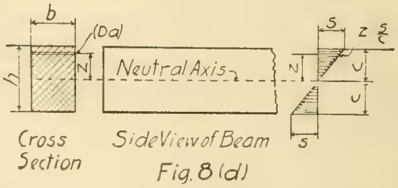


Fig. 8 *c* The three forces *X*, *Y*, and *Z* are indicated replacing the internal stresses (forces) in action in these beams to produce equilibrium in each beam. The detailed analysis is presented in paragraphs *29–37*.

Fig. 8 *d* Cross section and side view of any rectangular beam. To the right is a diagram showing the variation in the intensity of the horizontal internal stresses in such a beam. Explained in paragraph *38*.

31. Considering either part of the beam, a system of forces in equilibrium is seen, to which the three necessary and sufficient conditions of statics for forces in one plane apply:

Algebraic sum of all horizontal forces = 0
Algebraic sum of all vertical forces = 0
Algebraic sum of moments of all forces = 0

32. Regardless of the intensity and direction of these unknown stresses, let each be resolved into its horizontal and vertical components. The horizontal components will be applied at various points in the cross section, some in one direction and some in the other: that is, some of the horizontal stresses will be tensile and some will be compressive. But by the first condition above their algebraic sum is zero. The vertical forces will be added and form a resultant force V, which by the second condition equals the algebraic sum of the vertical forces on the left of the section. This vertical force V acts in opposite directions upon the two parts into which the beam is assumed to be separated, hence it is in the form of a shear.

33. Laws of internal stress. From a consideration of the foregoing, the following laws concerning the internal stresses in any section of any beam may be given:

1. The algebraic sum of the horizontal stresses is zero; the sum of the horizontal tensile forces is equal to the sum of the horizontal compressive stresses.

2. The algebraic sum of the vertical stresses forms a resultant shear which is equal to the algebraic sum of the external forces on either side of the section.

3. The algebraic sum of the moments of the internal stresses is equal to the algebraic sum of the moments of the external forces on either side of the section.

34. These laws are the foundation of the theory of the flexure of beams. *Resisting moment* is the term given the algebraic sum of the moments of the internal horizontal stresses with reference to a point in the section; 'bending moment' is the term for the algebraic sum of the moments of the external forces on either side of the section with reference to the same point (as for resisting moment).

35. 'Resisting shear' is the term applied to the algebraic sum of the internal vertical stresses in any section, and 'vertical shear' is the term for the algebraic sum of the external vertical forces on one side of the section.

36. Laws of beams. The foregoing principle may be summarized into the following three laws for any section of any beam:

1. The sum of the tensile forces = the sum of the compressive forces.

2. Resisting shear = vertical shear.

3. Resisting moment = bending moment.

37. Neutral surface and neutral axis. The above three theoretical laws do not furnish sufficient data for the full study of beams. Experiment and experience show that when a horizontal beam deflects one side becomes convex and the other concave. It is shown that the tensile stresses are on the convex side where the fibers have been elongated and compressive stresses are on the concave side where the fibers have been shortened. By experiment it is found that any two parallel vertical lines drawn on the beam before bending, remain straight after bending of the beam; but are nearer together than before on the compressive side and farther apart on the tensile side. These experimental laws may be stated:

4. The horizontal fibers on the convex side are elongated, and those on the concave side are shortened, while near the center there is a neutral surface which is unchanged in length.

5. The elongation or shortening of any fiber is directly proportional to its distance from the neutral surface. When the elastic limit of the material is not exceeded, the stresses are proportional to their changes in length; hence

6. The horizontal stresses are directly proportional to their distances from the neutral surface, provided all unit-stresses are less than the elastic limit of the material.

7. The neutral surface passes through the centers of gravity of the cross sections.

In order to make clear the application of these principles in the analysis of beams, the following simple demonstration is given.

38. Moment of inertia. In figure 8*d* let s = unit-stress on the horizontal fiber most remote from the neutral surface, at the distance c from the neutral axis.

From the sixth law above $\frac{s}{c}$ = unit-stress at the distance unity.

Then $z \cdot \frac{s}{c}$ = unit stress at any distance z from the neutral surface. Designating an elementary area by (Da) and its distance from the neutral axis by z then the resisting moment of the elementary area (Da) of the cross section, is equal to Da times the unit-stress $(z \cdot \frac{s}{c})$ on that area times the distance z from the neutral axis to this area or $(Da)\, z \frac{s}{c} \cdot z$. Designating the resisting moment of a single elementary area (Da) by the symbol (DM_R), then

$$\begin{aligned}(DM_R) &= (Da)\; z \frac{s}{c} \cdot z \\ &= (Da)\, \frac{sz^2}{c}\end{aligned}$$

The resisting moment for all elementary areas may be similarly expressed. It therefore follows that the total resisting moment for the entire area of the cross section will be the sum of all the resisting moments of all the elementary areas. This may be expressed by the following formula, in which Σ represents the adding together, or summation of all the resisting moments of the elementary areas,

$$\begin{aligned}\text{Sum of all } (DM_R) &= \text{sum of all } (Da)\, \frac{sz^2}{c} \\ &= \sum (Da)\, \frac{sz^2}{c}\end{aligned}$$

39. Since s and c are constants for any given case the above formula may be written,

$$\text{Resisting moment of the section} = \frac{s}{c}\, \Sigma\, (Da) \cdot z^2$$

The expression $\Sigma\, (Da).z^2$ is generally called the moment of inertia of the section considered, and is usually represented by the symbol I. Hence, the sum of the internal moments $\frac{s}{c}$ I, must equal the sum of the external bending moments about the section, and this relation is expressed by the following formula $\frac{s}{c}I = M$, in which the symbols indicate the following units:

s, pounds per square inch;
c, inches;
M, bending moment in inch-pounds at section;
I, biquadratic inches ($=$ inches^4).

40. By the use of this formula the maximum fiber stress may be found in any section of a beam, the cross section of which is bounded by straight or curved lines forming regular geometrical figures, provided the bending moment due to the external forces can be computed by the laws of equilibrium.

By a graphical method to be described later the principles just discussed may be applied without using the calculus for the determination of the moment of inertia.

41. The section modulus of any cross section of a beam is the measure of strength of the resisting moment and is equal to the moment of inertia divided by the distance from the neutral surface to the outermost fiber in the cross section.

$$\text{Section modulus} = \frac{I}{c}$$

42. Summary. Summarizing this discussion of the moment of inertia, the power of any elastic body to resist the action of forces which produce compression or bending stresses depends upon the following factors:

1. The physical properties of the material.
2. The shape of the cross section of the body.
3. The area of the cross section of the body.
4. The magnitude and direction of the external loads.
5. The manner in which the load is transmitted through the body.
6. The manner in which the body is supported.

Theory of column action

43. Factors affecting strength. The essential principles in the mechanics of columns are of importance in this study and will be very briefly considered. The strength of any column of homogeneous material of whatsoever shape or size is dependent upon the following factors:

1. The materials of which it is built.
2. The cross sectional area.
3. The shape of the cross section.
4. The ratio of the smallest diameter to total length of the column.

These factors are to a certain extent interdependent. It is obvious that different materials will vary in strength depending upon their physical qualities. If all other factors remain unchanged, an increase in cross sectional area will give increased strength.

44. The shape of the cross section is of the greatest importance. For example, a flat piece of card-board of rectangular form, if placed in a vertical position will carry only a very small weight applied to the upper end without bending. But if this same card-board be rolled into the form of a hollow cylinder, with the edges fastened together, it will carry without bending many times the weight previously carried by the flat sheet, although there has been no increase in the amount of material employed nor in the height of the column or sheet.

45. If the ratio of least diameter to total length of column is decreased, without decreasing the cross sectional area the stiffness of the column is reduced and its load-carrying capacity is reduced. In terms of mechanics the measure of stiffness of any column is expressed by the ratio of its length to its least moment of inertia.

46. Determination of moment of inertia. In the case of irregular cross sections such as are encountered in the bones, the determination of the moment of inertia can not be made by the integration formulas of calculus, but the methods of calculus may be applied graphically to the problem, and the moment of inertia of any section may be very closely approximated.

47. For such calculation an accurate drawing (tracing) is made of the cross section whose moment of inertia is to be computed. Then the principal axes, which are the axes of greatest and of least stiffness, are drawn. In most cases these are at right angles to each other and usually simple inspection will at once locate these axes, which intersect at the center of gravity of the cross section. Then a series of small squares are drawn or projected over the cross section with their sides parallel to the principal axes. In the study of the femur these lines forming the squares were drawn at intervals of $\frac{1}{20}$ inch, so that the area of each square is $\frac{1}{400}$ of a square inch. Then for each axis, a tabulation is made successively of the area of the cross section included between the adjacent lines parallel to the axis considered. Then each separate area thus found is multiplied by the square of the distance from the axis to the center of gravity of that particular area. Each product thus found is the moment of inertia of that area about the given axis. Each of the separate areas is multiplied by the square of the distance from the axis to the center of gravity of that particular area; and the sum of all these products is the moment of inertia of the cross section about that axis.

48. Example. For example it is required to determine the moment of inertia about the two principal axes of a rectangular figure 1.0 inch by 1.2 inches.

Let A–A and B–B denote the two principal axes as shown in figure 9. If the area is expressed in units of $\frac{1}{20}$ inch squares and the distances in terms of $\frac{1}{20}$ inch units, it will simplify the tabulations and the results may be accurately reduced to the usual units.

In the particular case assumed above, the exact integration by the formula derived from calculus may be used, and the graphical calculation of the moments of inertia about the two axes checked. For a cross section of rectangular shape the value of the moment of inertia is $I = \frac{bh^3}{12}$, where b is the breadth and h is the height or dimension of the section at right angles to the axis about which the moment of inertia is to be computed. Substituting in this formula we find for axis A–A, $I = \frac{1(1.2)^3}{12} = 0.1440$ inches^4. By the graphical method I for

TABLE 1

I FOR AXIS A–A					I FOR AXIS B–B				
(1) No. of strip 1/20 inch wide	(2) No. of 1/20 inch x 1/20 inch squares area	(3) Distance from Axis A–A to center of gravity of strip	(4) Square of column 3	(5) Moments of inertia column 2 x column 4	(1) No. of strip 1/20 inch wide	(2) No. of 1/20 inch x 1/20 inch squares area	(3) Distance from axis A–A to center of gravity of strip	(4) Square of column 3	(5) Moment of inertia column 2 x column 4
1	20	0.5	0.25	5.0	1	24	0.5	0.25	6.0
2	20	1.5	2.25	45.0	2	24	1.5	2.25	54.0
3	20	2.5	6.25	125.0	3	24	2.5	6.25	150.0
4	20	3.5	12.25	245.0	4	24	3.5	12.25	294.0
5	20	4.5	20.25	405.0	5	24	4.5	20.25	486.0
6	20	5.5	30.25	605.0	6	24	5.5	30.25	726.0
7	20	6.5	42.25	845.0	7	24	6.5	42.25	1,014.0
8	20	7.5	56.25	1,125.0	8	24	7.5	56.25	1,350.0
9	20	8.5	72.25	1,445.0	9	24	8.5	72.25	1,734.0
10	20	9.5	90.25	1,805.0	10	24	9.5	90.25	2,166.0
11	20	10.5	110.25	2,205.0		240			7,980.0
12	20	11.5	132.25	2,645.0		2			2.0
	240			11,500.0		480			15,960.0
	2			2.0					
480÷400=1.20 sq. in.				23,000.0	480÷400=1.20 sq. in.				15,960.0

$$I=\frac{23{,}000}{20\times20\times20\times20}=0.14375\text{ inches}^4$$

$$I=\frac{15{,}960}{20\times20\times20\times20}=0.09975\text{ inches}^4$$

axis *A–A* was found to be 0.14375 inches^4. The error by the graphical method is 0.00025, or one part in 576. For axis *B–B* similarly, $I = \frac{1.2(1)^3}{12} = 0.1000$: the value of I by the graphical method is 0.09975. The error by the graphical method is 0.00025, or one part in 400. These slight errors are far inside the permissible error for mechanical design.

49. Columns. Long slender columns tend to bend at the weakest cross section. If a column is rigidly braced against side movement at intervals along its length, its strength is greatly increased, because of the increase in stiffness, and the effect of such bracing is to make it in effect a series of short columns.

Horizontal shear in beams

50. Effect of horizontal shear in beams. The common theory of beams considers only the tension, compression and vertical shearing forces acting in a beam. In addition there exists a horizontal shearing force tending to cause sliding or shearing in horizontal planes parallel to the longitudinal axis of the beam.

The effect of these shears is seen in considering a cantilever beam loaded at the free end, the weight of the beam itself being neglected. A small amount of bending will take place in accordance with the relative stiffness of the beam. Figure 10, *a* shows such a beam, the dotted lines indicating the bending due to the load *P*. The amount of the bending is a maximum at the point where the load is suspended, and as the support is approached, the bending diminishes until it reaches a minimum of zero at the support.

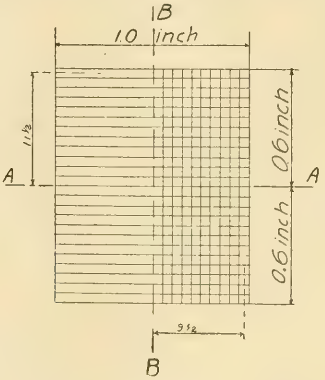


Fig. 9 Manner of subdividing any plane figure for the purpose of computing the moment of inertia, without the use of calculus formulas. Tabulations are given in table 1, explained in paragraph *48*.

If a beam having the same dimensions as the one just discussed, is considered, but in which horizontal cuts have been made through the beam, separating it into a number of thin strips as in figure 10, *b*, it is evident that the amount of bending in such a loaded beam will be much greater. This is because the stiffness of the beam has been reduced and each thin strip slides over the adjacent strip when the beam is loaded, as shown in figure 10, *c*. In the solid beam the stiffness is greater than in the beam of the same dimensions composed of a number of separate thin strips. This increased stiffness is due to the cohesion of the fibers which prevents the sliding of adjacent sections past each other. But the tendency of adjacent sections to slide is present and causes horizontal shearing stresses in every horizontal plane of the loaded beam.

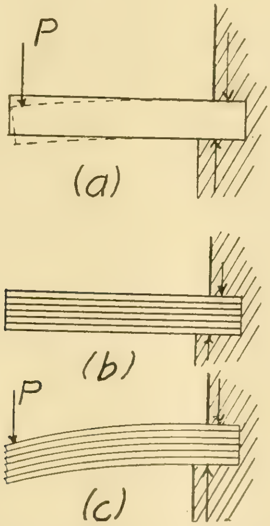


Fig. 10 Manner in which a vertical load produces horizontal shearing stresses in a loaded cantilever beam, explained in paragraph *50*.

From the application of the laws of equilibrium, the theory of beams and by the aid of the differential calculus the amount of the horizontal shear in any beam is given by the following formula:

$$S_h = \frac{V}{I \cdot t} \sum_z^c (Da) \, . \, z$$

in which V = Total vertical shearing force at section.

I = Moment of inertia of the cross section.

t = Thickness of the plane on which shearing takes place.

(Da) = an infinitesimal elementary area.

z = Distance of the shearing plane from the neutral surface in any section of beam.

c = Distance from neutral axis of beam to outermost fiber of beam.

$\sum_z^c$ = Sign of summation of all products for all values of z between c and z, as limits.

S_h = Intensity of horizontal shearing force in pounds per square inch.

The expression $\sum_z^c (Da).\, z$ is usually spoken of as the statical moment of the given cross section. It is the summation of all the products of the separate elementary areas by their respective distances from the neutral axis, for the area lying between two lines distant c and z, respectively, from the neutral axis.

The formula above given may be simplified to the following:

$$S_h = Q \cdot \frac{V}{A}$$

in which

V = Total vertical shearing force at section.

A = Total area of cross section.

Q = Factor by which the average vertical shear over the section is to be multiplied, in order to obtain the actual horizontal shearing force at the section.

$$Q = \frac{a_1 c_1}{r^2 t}$$

in which a_1 = area lying above the plane on which the horizontal shear is to be computed.

c_1 = distance from neutral axis to the center of gravity of the area, a_1.

$$r^2 = \frac{\text{Moment of inertia of section}}{\text{Total area of section}} = \frac{I}{A}$$

t = width of plane on which the horizontal shear is to be computed.

The successive sections of bone which will be studied are of such irregular shapes that the formulas of calculus can not be employed directly for the computation of the horizontal shears. For this reason graphical methods will be used which will give an accuracy within 1 per cent of the absolute values.

51. Computation of horizontal shearing stresses. To illustrate the methods that have been employed for the determination of the horizontal shearing forces, the computations in tabular form are presented for a circular cross section 2 inches in diameter (fig. 11, and table 2). Two axes at right angles are drawn through the center of the circle in figure 11. Then a series of lines is drawn parallel to axis A–A at intervals of $\frac{1}{20}$ inch, and a second series is drawn at right angles to A–A, thus dividing the area above A–A into squares whose sides are $\frac{1}{20}$ inch long. The strips are numbered in order from the axis A–A, the first being 1, and so on, as shown in column 1, table 2. In column 2 are given the areas of the successive strips. Column 3 gives

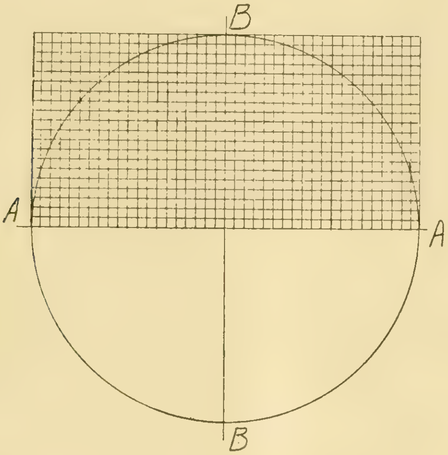


Fig. 11 Circle two inches in diameter, subdivided into strips $\frac{1}{20}$ inch wide for the purpose of computing the shear factors by which the average vertical shear is multiplied, to give the value of the horizontal shearing stress at any given point in a beam of circular cross section. See paragraph *51*.

the distance from the axis A–A to the center of gravity of each strip. Column 4 gives the product of each area by its distance from the axis. Column 5 gives the static moment of all the areas that lie beyond the strip, opposite which the static moments are tabulated. Column 6 is the width or thickness of the shearing plane as scaled from figure 11. Column 7 gives the products of r^2, a constant for a given cross section, by t; for a circular cross section $r^2 = d^2/16$. If d (the diameter) is expressed in twentieths of an inch, we have $r^2 = 40^2/16 = 100$. Hence $r^2t = 100t$. Column 8 gives the values of Q, the factor by which the average vertical shear at any cross section must be multiplied to give the amount of the true horizontal shear

TABLE 2

Computation of shear factors, Q, for a circular cross section, 2 inches in diameter

(1) No. of strip parallel to axis A-A	(2) Area of strip in units of 1/20 inch x 1/20 inch	(3) Distance from neutral axis A-A to center of gravity of strip	(4) Static moment of strip col. 2 x col. 3 . (1/20 inch)	(5) Static moment $\sum_{z}^{c}(Da)z = a_1 c_1$	(6) t = thickness of shearing plane	(7) $r^2t = 100.t$	(8) Q = coefficient of horizontal shear. Col. 5 ÷ col. 7
0				5286	40.0	4000	1.322
1	40.00	0.5	20				
2	39.75	1.5	60	5206	39.75	3975	1.312
3	39.50	2.5	99				
4	39.25	3.5	137	4970	39.00	3900	1.276
5	38.75	4.5	175				
6	38.25	5.5	210	4585	38.00	3800	1.206
7	37.75	6.5	245				
8	36.75	7.5	276	4064	36.25	3625	1.122
9	35.75	8.5	304				
10	35.00	9.5	333	3427	34.25	3425	1.001
11	33.75	10.5	354				
12	32.25	11.5	371	2702	32.00	3200	0.843
13	31.00	12.5	387				
14	29.25	13.5	394	1921	28.00	2800	0.687
15	27.25	14.5	395				
16	25.00	15.5	387	1139	24.00	2400	0.474
17	22.25	16.5	367				
18	19.75	17.5	348	424	18.00	1800	0.236
19	14.25	18.5	263				
20	8.25	19.5	161		0.00		0.000

at the given point. These values of Q hold for any circular cross section. As the cross section is symmetrical about A–A (fig. 11) the factors Q are computed for only one-half of the cross section.

A similar application of the theory of horizontal shear will be made for various cross sections of the femur to be taken up in a subsequent section.

52. Vertical shearing stress. The true vertical shearing stress at any point in a beam acts at right angles to the direction of the horizontal shearing stress, and has the same numerical value.

53. Lines of stress in beams. In the preceding section it was shown that at any point in a beam there is a horizontal and a vertical shearing stress, S_h, the formula for which was also given (*50,* p. 206).

At the same point there is also a longitudinal compressive or tensile unit stress, S, which can be computed from the beam formula, $S = Mc/I$, and the principle that these longitudinal stresses vary in proportion to their distances from the neutral axis.

It can be shown that these unit-stresses combine to produce maximum and minimum normal stresses on planes at right angles to each other, and maximum shearing stresses on the planes that bisect these planes.

From mechanics it is found that the angle θ, which the direction of a maximum or minimum normal tensile stress makes with the neutral surface is given by the following formula,

$$\text{cotangent } 2\theta = \frac{\frac{1}{2}S}{S_h}$$

and the magnitude of this tensile stress is given by the formula,

$$S_n = \tfrac{1}{2}S + \sqrt{S_h^2 + (\tfrac{1}{2}S)^2}$$

If the minus sign be placed before the radical, this formula will give the amount of the compressive stress at right angles to the maximum tensile stress at the same point.

In the above formulas

S_n = normal tensile stress (maximum or minimum).
S_h = horizontal shearing unit-stress.
S = longitudinal tensile unit-stress.

The same formulas may be applied to the determination of the magnitudes and directions of the maximum or minimum normal compressive stresses.

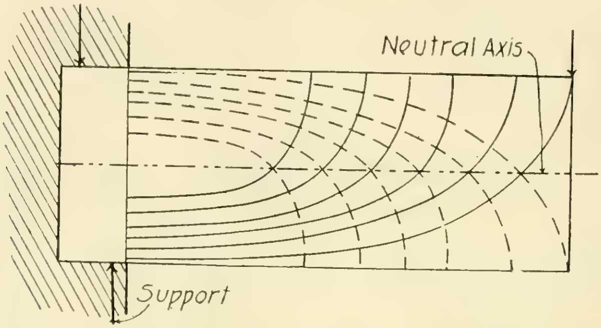


Fig. 12 *a* Typical paths of the lines of maximum stress in a cantilever beam of same over-hang and average size as femur analyzed in Part III. (See figure 14.)

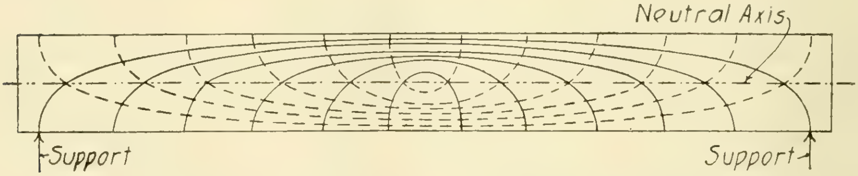


Fig. 12 *b* Typical paths of the lines of maximum stress in beams on two supports.

By the use of these formulas the magnitudes and directions of the maximum and minimum tensile and compressive stresses may be computed for a large number of points in the beam, and then curves may be drawn showing the paths of the maximum tensile and compressive stresses throughout the length of the beam.

Figure 12 *b* is a side view of a simple beam resting on two supports, for which a number of the lines indicating the directions of the maximum tensile and compressive stresses have been drawn. The lines of maximum compression are shown as solid lines and those of maximum tension are indicated by dotted lines. Figure 12*a* is a similar drawing to represent the lines of maximum stress in a cantilever beam; the same notation is used in both figures.

The lines of maximum stress shown in figure 12*b* exist in every loaded beam on two supports; while in loaded cantilever beams somewhat similar lines also exist. The direction of the lines depends somewhat on the manner in which the loads are applied to the beam, and the amount of these maximum stresses, of course, varies with the amount of the load. The general formulas given for the determination of the amounts and directions of these maximum stresses hold true for all beams. The internal strength of the beam must be exerted in the paths of these maximum stresses in order to oppose and balance them. There is an infinite number of these lines of stress, of which those drawn in figs. 12*a*, *b* are only a small part, but whose shape and position is characteristic. The cohesion of the fibers of the beam, its shearing strength and its resistance to compression and to tension combine in the beam to resist the maximum stresses acting in these peculiar curved paths. The beam would act a great deal more efficiently if the material were laid down in curved lines pursuing the same paths as the lines of maximum stress: in this manner considerably less material would be required to support the same external load.

In Part III, the lines of maximum tensile and compressive stress will be drawn in a similar manner in the femur, after computation of their magnitude and direction, by applying the principles and methods of mechanics discussed in this section.

Properties of bone

In order to investigate the structure of bone from a mechanical point of view, it is necessary to determine the physical properties of this material and to have proof that it obeys similar laws to those that obtain for other materials such as wood, iron, steel, concrete, etc.

Through experiment and the testing of sections of animal and human bones in testing machines it has been fully demonstrated that they act as other elastic materials (wood, steel, etc.) and therefore the same laws of mechanics may be applied to their study and the determination of their strength.

Rauber ('76) has made very complete studies of the behavior of sections of bone taken from various parts of the skeleton, by testing them in various ways to determine the ultimate stresses that small sections actually develop.

1. Specific gravity. Hülsen ('98) gives the specific gravity of fresh, compact human bone from 1.507 to 2.024.

Rauber gives the specific gravity of fresh compact bone of femur and tibia as follows:

Man 30 years old	1.901
Woman 56 years old	1.825
Cat	2.101
Bullock	2.024
Calf	1.889
Domestic hog	1.965
Wild hog	2.060

He also gives the specific gravity of fresh spongy bone from the human femur head, 1.197.

I have made experiments carefully on sections of compact bone from the shaft of human femurs as found in the dissecting room, which gave variations from 1.915 to 1.990 in a series of 17 specimens, with an average of 1.955. These figures are based on bone from which the marrow, blood and other non-osseous material had been removed by boiling and then air drying for a number of days.

2. Tensile strength. The ultimate tensile strength of fresh, normal, compact, human bone is variable between 9.25 and 12.41 kilograms per square millimeter, or in English measure from 13,000 to 17,700 pounds per square inch, according to Rauber's tests. Hülsen ('98) gives the ultimate tensile strength of similar bone as 10.40 to 10.56 kilograms per square millimeter or from 14,750 to 14,980 pounds per square inch.

3. Compressive strength. The ultimate compressive strength of such compact bone is variable from 12.56 to 16.85 kilograms per square millimeter, or from 18,000 to 24,000 pounds per square inch according to Rauber's tests. Hülsen gives as the ultimate compressive strength of similar bone 18.95 kilograms per square millimeter, or 26,850 pounds per square inch.

The disproportion between the strength of compact bone in tension and in compression seems to be general among the vertebrates, as indicated by the following data, by Rauber:

	ULTIMATE STRENGTH		RATIO
	Tensile	Compressive	
Fresh compact bone, man	12.41	16.85	0.73
Fresh, compact bone, bullock	11.46	13.31	0.86
Fresh, compact bone, calf	8.93	12.26	0.73
Dried, compact bone, hog	7.30	11.73	0.62
Dried, compact bone, wild hog	10.29	14.71	0.70

Figures are in kilograms per square millimeter.

4. Shearing strength. The shearing strength of compact, human bone at right angles to the long axis of the bone is 11.85 kilograms per square millimeter, or 16,800 pounds per square inch: the shearing strength of such bone parallel to the long axis of the bone is 5.03 kilograms per square millimeter, or 7150 pounds per square inch (Rauber).

5. Modulus of elasticity. Rauber gives the modulus of elasticity of fresh, compact bone of the femur of a 46-year old man as 1982 to 2099 kilograms per square millimeter, or 2,820,000 to 2,980,000 pounds per square inch. He also gives as the modulus of elasticity for compact bone of the human tibia 1871 to 2041 kilograms per square millimeter, or 2,420,000 to 2,910,000 pounds per square inch.

6. Torsional strength. The ultimate torsional strength of compact bone varies, according to Rauber, from 4.0 to 9.3 kilograms per square millimeter, or from 5700 to 13,300 pounds per square inch. The ultimate torsional strength per square inch for timber is 2000, cast iron 30,000 and steel 65,000 pounds per square inch.

7. Comparison of bone with other materials. The subjoined table giving the strengths of various building materials, is derived from the figures published in standard structural handbooks. The figures for the strength of human tissues is derived from Rauber's work.

TABLE 3

Strength of bone compared with other materials

SUBSTANCE	WEIGHT IN POUNDS PER CUBIC FOOT	MODULUS OF ELASTICITY TENSION AND COMPRESSION	ULTIMATE STRENGTH. POUNDS PER SQUARE INCH				SPECIFIC GRAVITY
			Tension	Compression	Shear	Modulus of rupture	
High steel	490	3,0000,000	130,000	120,000	50,000	100,000	7.9
Medium steel	490	30,000,000	65,000	60,000	40,000	60,000	7.9
Wrought iron	480	28,000,000	50,000	55,000	40,000	50,000	7.6
Cast iron	450	15,000,000	25,000	90,000	18,000	35,000	7.2
Platinum	1,325		48,000				21.2
Silver	657		41,000				10.5
Gold	1,208		38,000				19.3
Bronze	500		30,000	53,000		56,700	8.0
Brass, low	506		9,000	52,000			8.1
Brass, high	543		32,000	120,000			8.7
Copper	562		28,000	42,000		29,800	9.0
Zinc	443		5,400	22,000		7,500	7.1
Tin	457		3,500	6,400		3,700	7.3
Lead	706			7,000			11.3
Granite	170	7,000,000	1,500	15,000	2,000	1,500	
Limestone	170	7,000,000	1,250	6,000	1,000	1,200	
Sandstone	150	3,000,000	750	8,000	1,500	1,200	
Brick	125	2,000,000		4,000	1,000	600	
Concrete 1, 2, 4	150	2,500,000	300	3,000			
Organic materials:							
Oak, white	46	1,600,000	*12,500	*7,000	*800	7,200	0.73
			†2,000	†2,000	†4,000		
Pine yellow	44	1,500,000	*12,000	*7,000	*600	7,200	0.70
			†600	†1,400	†5,000		
Pine white	30	1,000,000	*7,000	*5,500	*400	4,200	0.48
			†500	†800	†2,000		
Compact bone (low)	119	2,900,000	*13,200	*18,000	†11,800		1.91
Compact bone (high)			*17,700	*24,000	*7,150		
Human tendon			9,850				
Human cartilage			2,250	3,900			
Rib cartilage			240	2,250			
Nerve			1,300				
Artery			230				
Vein			180				
Muscle			77				
Ivory			17,000				

* Indicates stresses with the grain, i.e., when the load is parallel to the long axis of the material, or parallel to the direction of the fibers of the material.

† Indicates unit stresses across the grain, i.e., at right angles to the direction of the fibers of the material.

PART III. THE MATHEMATICAL ANALYSIS OF THE FEMUR

Introduction

The work of Wolff in developing the theory of the functional form of bone has been of immense value and marks a great advance in the surgery of the bones. His theory was based on the study of the femur in great detail, the anatomical relations being carefully described, and the deductions based on the normal structure were supplemented by a large number of pathological specimens of bone which were considered in much less detail than the normal femur.

1. The Culmann model. The foundation for his whole theory, however, was the mathematical analysis which Culmann made of a model of the head and neck of the femur. This model (fig. 13) had a circular cross section and the diameter varied gradually from a maximum at the head to a minimum in the shaft as shown. The analysis of this model was made for a load of 30 kilograms placed on the head which acted in a direction parallel to the shaft. The lines of maximum stress in this model were calculated on the supposition that the model was a solid body. Under the assumed conditions the analysis of the lines of maximum stress in the model is correct. But it must be pointed out that the assumed model does not resemble the normal femur, except remotely, and therefore the assumption that the lines of stress in the model explain the position of the trabeculae in the head of the femur is not warranted.

2. Mathematical objections to Culmann's conclusions. Without disparagement of Wolff's or Culmann's work the following objections may be fairly urged against the soundness of their mathematical analysis of the head of the femur:

1. Only a small part of the femur was analyzed and the relation of this part to the whole bone as a structure was not shown.

2. The direction in which the assumed load on the femur acted was taken parallel to the shaft of the femur. This point will be considered in greater detail under the heading, Loading, in Part III.

3. The model of the femur on which the analysis was made was of such a shape that the large mass comprising the greater trochanter was entirely omitted.

5. The mathematical analysis of the model, although strictly correct for the model, can not be accepted as holding true for the femur which has a decidedly different shape from that of the assumed model.

Fig. 13 Culmann's model of a Fairbairn crane, with the lines of internal stress computed for a load of 30 kilos as shown.

6. The quantitative relations between the load and the areas of the cross section of the femur have not been determined.

I believe that the foregoing objections are largely responsible for the rejection of Wolff's theories by some investigators and the misinterpretation of the true significance of the inner structure by many others.

The basis of a mathematical analysis of the femur

Freiberg ('02) has urged that to establish a satisfactory mathematical proof of the mechanics of the femur "we must know every possible stress to which the bone is to be submitted under normal conditions, and these stresses must be expressed in figures." This statement may appear fair and logical but it demands an excessive amount of evidence. No engineering structure of even the greatest magnitude would be designed in accordance with the strict requirement above quoted, that every possible stress must be known. In the development of any structural design the object sought is to compute the maximum effects of all loads to be carried by the structure, and the various combinations of these forces which could be in action at one time to produce maximum effects. But no attempt is made to consider the effect of every possible stress.

For example, in the design of a railroad bridge the general outline or skeleton form of the structure is assumed; then the stresses are computed in each part of the structure which are produced by the dead weight of the structure (which must be assumed), the live or moving load that produces maximum effect, the weight of snow, the effect of impact due to rapidly moving loads, and the force of the wind. These stresses are computed separately for each factor causing stress and then the maximum stresses in each part are determined by adding together the stresses caused by the combinations that obviously can or may act simultaneously on the structure. Then each part is proportioned to safely carry the maximum stresses that occur in it, under the assumed conditions.

But were we merely striving to determine if a certain structure were designed in accordance with the principles of mechanics the procedure would be reversed. The form of the structure would be known and its dimensions in every part. The stresses produced by the dead weight of the structure could be readily calculated from the principles of mechanics. A comparison of the stresses produced by the dead load with the strength of each part of the structure would show in practically every case a fairly close agreement, or parallelism, which would be conclusive that the parts were proportioned in accordance with mathematical laws. Further study of the design would show the amount of moving load, etc., that could be safely applied to the structure.

In the femur the body weight in the standing, walking or running positions produces preponderant stresses, which are much greater in magnitude than normally may be produced by the muscles alone. For example, in walking it will be shown that the maximum stress in the neck of the femur is 2098 pounds per square inch and in running it is 4192 pounds per square inch in the specimens analyzed in this paper. As the ultimate strength of muscle is about 77 pounds per square inch and it has practically no compressive strength, it is evident that the rôle of the muscle as affecting the inner architecture of the femur must be secondary to that of the static loads transmitted through the femur-head due to the superincumbent body weight. As the mechanical effects produced by the body weight acting through the femur-head are undoubtedly far greater than those normally due to muscle action, the structure of the femur must be designed to resist these maximum stresses due to body weight.

For these reasons, in this study no attempt will be made to analyze the effect of various muscles, or combinations of muscles, as affecting the inner architecture. A more detailed discussion of such effects is given in a subsequent paragraph (p. 224).

The mechanism of human locomotion

The modern views of this subject began with the publication of Borelli's classic (1681). Little further progress in the study of human locomotion was accomplished until the appearance of the epoch-making work, Die Mechanik der menschlichen Gewerkzeuge, by the Weber brothers in 1836. Their ingenious and original researches firmly established the mechanism of muscle action upon a firm scientific foundation. Improvements in the methods of study have been produced by the extended use of graphics and the development of instantaneous photography. Carlet ('72) and Marey ('72) developed graphic methods of great value and the study of successive phases of movements in man by instantaneous photography, first applied by Muybridge ('87), and later by Marey ('94) and others, have produced very valuable results in the study of human locomotion.

The Webers have shown that in both walking and running the body is thrown forward by rhythmic and alternating muscle contractions, chiefly of the lower limbs. In walking one foot is always in contact with the ground, but in running after one foot has touched the ground the body is projected into the air for a brief interval and the succeeding contact with the ground is made with the other foot.

In walking the step begins with placing the foot on the ground, the foot and the knee being in moderate flexion, the weight of the body falls on the heel of the forward foot and at the same instant the great toe of the other foot is in contact with the ground. At the next instant, through the action of the muscles of the lower leg additional pressure is applied through the great toe of the foot behind and the body is thrust forward. Then the foot behind is drawn forward and flexed simultaneously and swung forward to pass the loaded foot. Just as the swinging foot passes the loaded foot the weight of the body is transferred to the ball of the foot and just before the swinging foot touches the ground the weight on the loaded foot is transferred to the great toe. As the forward foot strikes the ground the weight of the body is borne by the great toe of the rear foot and the heel of the forward foot, and a sudden thrust from the great toe propels the body forward and the rear foot rises from the ground. The greatest pressure on the ground occurs when the thrust is given by the great toe of the rear foot, and the amount of this maximum pressure as determined by Carlet with exploring shoes which communicate the pressure on the foot through tambours, which automatically recorded the pressures, is a little greater than the body weight but never exceeds 44 pounds. Most of this increment of pressure above the body weight must be produced by the muscles of the lower leg and foot and therefore no account of it need be taken in the analysis of the stresses in the femur due to walking.

In running there is greater extension of the leg and the forward inclination of the body is greater than in walking. The succession of muscular movements in running is essentially the same as in walking but there is greater intensity of muscle action and the phases differ greatly in relative duration. While the body is suspended in the air between the periods of contact with the ground, both legs swing forward one behind the other. As contact is made with the forward foot the other swings past and there succeeds a quick forward thrust of the foot behind and then for a brief interval the body is in the air. The duration of the contact of the foot with the ground is shorter in running than in walking and the greater the force of contact the shorter the time of contact. The length of time the body is in the air is variable, increasing slightly with the speed of running.

Marey ('82) has shown that the maximum vertical rise in the position of the body occurs just as one foot reaches the ground. When running the leg bearing the weight is flexed about 30 degrees at the knee and the body is flexed at about the same angle at the hip according to the chronophotographic plates of Marey. No exact figures have been thus far published giving the relation of the maximum pressure to the body weight in running, but it is certain that the maximum effect must be produced shortly after contact has been made by one foot. Another factor to be considered is the tendency to cushion the impact of the foot as it strikes the ground in running. This effect is produced by the flexion at hips and at the knee of the loaded leg as described above, together with the sudden tensing of ligaments and muscles as the impact between foot and ground occurs. The action in some respects is very similar to that of certain types of shock-absorbers used on automobiles.

In the absence of absolute values of the amount of pressure produced by the sudden contact of the foot in running, it appears entirely rational to consider the effect to be the same as a load equal to the body weight applied suddenly to the foot. The mechanical effect of a suddenly applied load is exactly double that of a static (stationary) load of the same amount. That is, the stresses produced in a structure by a load, of say 100 pounds, suddenly applied, will be as great as those produced by a static load of 200 pounds.

Obviously the stresses in the femur due to running will be greater than those due to walking. In running or in walking the actual weight carried by the head of the femur which is loaded, is the body weight less the weight of the loaded leg itself (20 per cent of the body weight) or a load equal to 80 per cent of the body weight. The effect of running is to produce stresses in the femur equal to those produced by twice as great a static load as the weight borne on the femur-head. Hence the stresses in the femur due to running are those produced by a static load of 160 per cent of the body weight. This will be considered in later paragraphs discussing the factor of safety in the femur (p. 274).

Material studied

A preliminary study of the architecture of the femur and other bones was made, utilizing femurs and other material from the dissecting-room. This work yielded considerable information as to the planes in which sections should be cut in order to show most clearly the essentials of the inner architecture of the bones studied. Some 25 or more femurs were studied in this manner, but as nothing definite was known regarding the subjects, state of health at death, body weight, etc., no detailed quantitative analysis of such specimens was undertaken.

If quantitative relationships are to be established between bone structure and body weight, the specimens analyzed must be those which represent conditions that obtain in the normal, healthy individuals. The femurs finally obtained for such a study were those of an active, healthy American negro who had been killed accidentally. This subject was a large-framed, well-nourished laborer, about 35 years old, six feet in height and weighed 200 pounds. At the time of death he was in splendid physical condition.

It is evident that the inner architecture of the femurs from such a subject truly represents the structures in the normal living individual. For this reason, it is believed that the data derived from this study represent the true quantitative relations and actual mechanical conditions that exist in the normal individual. It is recognized that individual variations occur, depending upon age, sex, occupation, body weight, etc.

The data derived from the study of a single specimen are always subject to the criticism that it was unusual in some respect. In order to be as certain as possible that the shape and general arrangement of the inner architecture of the specimens analyzed were not exceptional, they have been compared with about 30 femurs obtained from the dissecting-room. From such comparison of the general form of the bone and of the inner structure of various longitudinal and cross sections made through similar planes in the specimens examined, I am certain that the two femurs (from the same subject) analyzed in this paper are typical, normal femurs.

Longitudinal serial frontal sections were made of the left femur and serial transverse sections of the right one, the details of which will be taken up in subsequent paragraphs.

The femur as a structure

1. *General.* It has been shown in Part II that human bone when subjected to loading acts as do other elastic bodies, and therefore, it conforms to the laws of mechanics which govern the action of elastic bodies under stress.

From a mechanical point of view the femur is seen to be a long, slender column whose upper extremity is bent at a considerable angle to the shaft. Figure 14 is an accurate outline drawing of a longitudinal section through a normal femur, cut in such a manner as to pass through the axis of the bone and in a plane approximately vertical, the femur being considered in the position normally assumed in the standing, erect attitude.

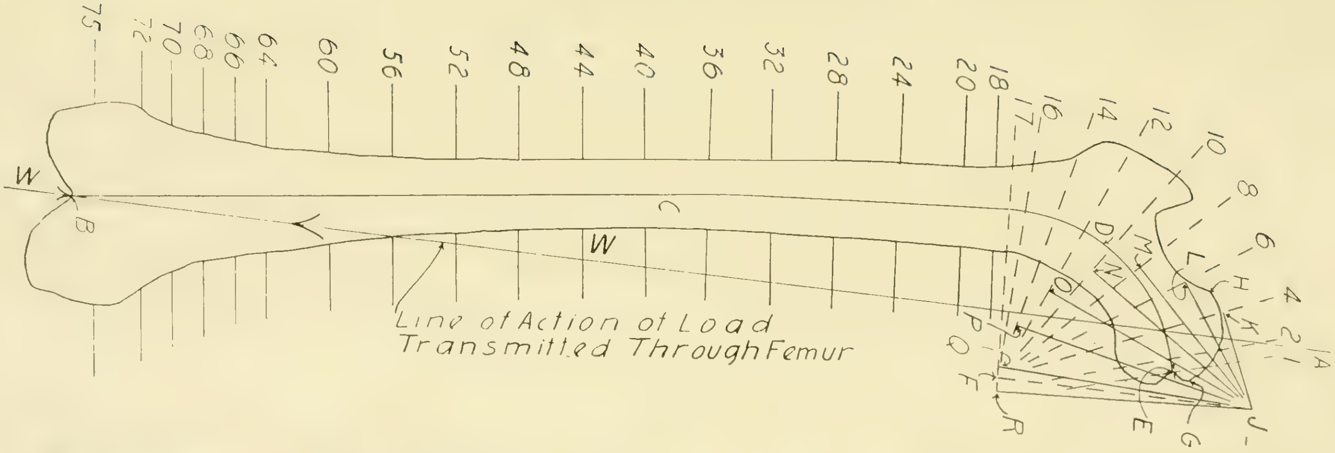


Fig. 14 Force diagram of the normal femur. In this diagram the exact outline of the femur, cut in longitudinal frontal section, was obtained from the left femur of the same subject, whose right femur is analysed in detail in this paper. The diagram shows the normal position of the line of action of the body weight transferred through the femur, and the positions of the transverse sections analyzed are indicated by numbered lines. See discussion of 'Force diagram,' Part III. The numbered sections show the positions of the corresponding numbered transverse sections of the right femur, which are given in full size in Plates 1–5.

In this figure no attempt is made to show the internal structure, merely the outline of the femur in the plane described is given.

In order to compute the internal stresses in such a structure it will be necessary first to consider the external loads that produce these stresses.

2. External loads. A. The effect of muscle action. It may be taken as axiomatic that muscles act almost entirely in tension. On the contrary, bone has great strength in resisting both tension and compression. The chief function of the bones from the mechanical point of view is to serve as supporting structures, the stresses in bones being chiefly compressive.

In the femur, the difficulty of taking into consideration the effect of the action of the muscles is, first: the wide variations in the amount of the forces exerted by them, and second, the relative effect of muscle action is small as compared with the load on the femur when the weight of the body rests on the femurs either in the standing or walking positions. The tensile strength of compact bone is about 230 times as great as that of muscle for similar areas of cross section. The net area of cross section of a normal femur may be taken roughly as about 0.80 square inches of compact bone. To produce sufficient tension by the action of the muscles to develop the full strength of the femur there would be required about 170 square inches of cross section of muscle. As the actual cross sectional area of the muscles of the thigh at about the middle of the shaft is about 25 square inches, the greatest possible action of the muscles could develop only one-seventh of the strength of the femur. Furthermore, maximum tension in the muscles of the thigh takes place in general groups, the flexors alternating in action with the extensors, so that in reality only about half of the area of the muscles is in maximum tension simultaneously. This would develop only about one-fourteenth of the maximum strength of the femur.

From the foregoing it is evident that, though it is recognized that the action of the muscles exerts an appreciable effect on the stresses in the femur, it is relatively small and very complex to analyze. For this reason the effect of the action of the muscles will not be investigated further in this study.

B. The effect of the body weight. The center of gravity of the body in the normal, erect, standing position passes slightly posterior to the plane passing through the centers of the heads of both femurs, and slightly anterior to the centers of bearing at the knee-joint. This is readily verified by a study of the relations of the ilio-femoral ligament, which holds the weight of the body balanced upon the heads of the femurs; and by the great preponderance of tendon strength at the knee-joint which is arranged centrally and posteriorly to the center of gravity of the lower end of the femur. The eccentric position of the center of gravity line of the body with respect to these two joints is one of considerable mechanical importance; excessive or suddenly applied loads reaching the femur through the acetabula are cushioned by the tensing of the muscles and ligaments at these joints.

In the standing position the weight of the trunk is transmitted in a vertical line passing through the acetabulum and the outer condyle. So the erect, standing position is maintained not only by the approximation of the bones, as would be the case if the perpendicular line through the acetabulum passed in succession through the center of the knee-joint, the axis of the tibia and the center of the ankle-joint, but also by the action of the muscles and ligaments at these joints. This action of the muscles and ligaments at these joints on the medial side brings the resultant pressure line in the leg to pass close to the centers of the knee- and ankle-joints. The eccentricities at these joints vary in different individuals and usually produce slight effect upon the principal stresses that normally occur in the femur. For this reason they will not be considered in the analysis of the loads.

3. Magnitude and direction of the external load. The weight of the body acts in a vertical direction and in the erect, standing position is equally divided between the two femurs. In walking and running the whole body weight is alternately transferred from one foot to the other, the center of gravity of the body being shifted laterally at each step so as to pass through the acetabulum and the centers of the knee- and ankle-joints. If the weight of one leg be taken equal to 20 per cent of the body weight, the load on each femur-head in the standing position will be approximately 30 per cent of the body weight. In walking and running the weight transmitted through the loaded femur would be the weight of the trunk (60 per cent) and of the lifted leg (20 per cent), or 80 per cent of the body weight.

The direction of the load transmitted through the femur, as has been shown above, can be taken as the straight line joining the center of the head of the femur to the center of gravity of the lower end of this bone. This line of action of the load is indicated by the line *AB* in figure 14. The line of action of the load is the line joining the point of application, at which the applied load may be regarded as concentrated, to the point through which the supporting force at the opposite end may be regarded as concentrated. The load is actually transferred from section to section through the bone from the uppermost to the lowermost extremity of the femur.

4. Assumed load on the femur. All calculations will be based upon a load of 100 pounds applied to the head of the femur and acting in the direction just indicated. This is a convenient basis of making the calculations and it facilitates percentage comparisons, while the effects produced by any other load may be accurately determined by simple proportion.

5. Force diagram. Figure 14 is a diagram of the longitudinal section through the axis of the femur showing the outline of the bone and the position of the line of action *AB* of the external load. This section does not lie strictly in a single plane, but follows the slightly curved axis of the bone which has its convexity directed anteriorly. As the head of the femur is directed about 12 degrees forward and medially to the frontal plane, this longitudinal section bears the corresponding relation to the frontal plane.

In figure 14 an axis *BCDE* is shown which is drawn so as to pass through the center of gravity of successive transverse sections of the femur. It is, therefore, the neutral axis of the femur. The distal 2 inches of this axis are formed as the prolongation of the axis of the shaft in order to simplify the figure, no inaccuracy being involved thereby.

Analysis of the mechanical properties of the femur

1. Analysis of serial transverse sections. Serial transverse sections were cut from the right femur in the following manner. An approximate longitudinal axis was drawn on the anterior surface of the femur so as to lie midway between the lateral and medial outlines of the bone when viewed from the anterior. The position of this axis was approximately that indicated by the axis *BCDE* in figure 14. Then at intervals of $\frac{1}{4}$ inch, measured along this axis, lines were drawn perpendicular to it to mark the positions of the transverse sections. In the curved portion of the upper femur the dividing lines were drawn perpendicular to the curve at successive points. As these dividing lines in the curved portion intersected near a common point, *F*, (fig. 14) this was taken as a common intersection of all these lines except the second line, marked *2* in figure 14. In this figure only the alternate dividing lines of the curved portion are shown, and are indicated by *2*, *4*, *6*, etc.

The serial sections thus marked out were sawed in planes perpendicular to the plane of figure 14, and passing through the division lines marked at the $\frac{1}{4}$-inch intervals. In the curved portion of the upper femur the method adopted in sawing the sections was as follows: The first saw-cut was made through line *1* (fig. 14), then a thin section was cut parallel to the first cut; the next cut was made through the line marked *2*, thus cutting a wedge-shaped piece. The next saw-cut was made parallel to the cut through line *2*, making a thin section of uniform thickness. This procedure was continued until the straight part of the shaft was reached, after which the sections were cut at intervals of $\frac{1}{4}$-inch until the expansion of the lower femur was reached at section 64. From this section to the lower end of the femur thin sections were cut as in the head and neck of the femur, except that the thicker sections were of uniform thickness.

Of the 75 transverse sections into which the femur was cut as just described, the following sections were analyzed in detail and are shown in Plates 1 to 5: 2, 4, 6, 8, 10 12, 14, 16, 18, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 66, 68, 70, 72, and 75. The positions of these sections are indicated by these numbers on the various drawings and diagrams, which also indicate the distances in $\frac{1}{4}$-inch units from the upper extremity of the femur to the corresponding sections, as measured along the longitudinal axis. The detailed study of these sections will now be taken up.

2. Center of gravity. The first step in analyzing these transverse sections is the accurate location of the center of gravity of each section. The true longitudinal axis is the line joining in succession the centers of gravity of the transverse sections.

The center of gravity of each section was located by the method of suspension. The transverse section whose center of gravity is to be determined is suspended from any point and the direction of the suspending thread is carefully marked on the section. The operation is then repeated, suspending the section from any other point, and marking on the section the direction of the suspending thread. The center of gravity of the section lies at the intersection of the two lines whose directions have been marked on the section. A third line may be found in a similar manner, and this should pass through the point of intersection of the other two lines, thus checking the accuracy of the determination. Every section has been twice checked in this manner. In each of the sections shown in Plates 1–5 the position of the center of gravity is at the intersection of the two axial lines *A–A* and *B–B*.

3. Neutral axis and neutral surface. As has been more fully explained under the same heading in Part II, the neutral surface of a beam is that surface or plane on which there is neither compressive nor tensile stress. This surface passes through the center of gravity of the section considered. This surface is approximately at right angles to the vertical plane passing through the head and neck of the femur when the body is in the normal erect position of ‘attention.’

The neutral axis *BCDE,* figure 14, is drawn to pass through the maximum number of centers of gravity of the transverse sections. The few exceptions where it fails to pass through the center of gravity occur where the cross section undergoes a marked change in shape and size with consequent distortion of the position of the neutral axis.

4. Moment of inertia. The graphical method of determining the moment of inertia has been used throughout as described in Part II (*46–48*). The two axes about which the moments of inertia have been calculated are at right angles. The axis which is of chief importance in the analysis of loads on the femur-head is indicated by *A–A* in Plates 1–5, and in all tables and diagrams. This axis lies in the neutral plane.

The difference in the consistency of the bone in the various sections is properly allowed for in the following manner: Where the section is entirely composed of cancellated bone the moment of inertia is computed as for compact bone having the same size and shape. The section is then carefully weighed and the ratio of the weight of the cancellated bone to compact bone of the same area and thickness is determined, this ratio being called the ratio of consistency of the cancellated bone. The effective moment of inertia is then found by multiplying the moment of inertia of compact bone by the ratio of consistency of the cancellated bone. This is the effective moment of inertia of that section in terms of compact bone.

Where a section is composed of both compact and cancellated bone, the area of each is determined, and the moment of inertia is computed separately. The section is carefully weighed. The weight of the compact bone is computed for a section of the same area and thickness, and this is subtracted from the total weight of the section, giving the weight of the cancellated bone in the section. The ratio of the weight of the cancellated bone to the weight of compact bone of the same area and thickness is found, and is the ratio of consistency as previously defined. The effective moment of inertia of the cancellated bone in the section is equal to the product of the moment of inertia of the cancellated bone by its ratio of consistency. The total effective

TABLE 4

Computations for transverse section No. 8. Moment of inertia, coefficients of horizontal shear and horizontal shearing unit-stresses. Horizontal shear unit-stresses for 100-pound load on femur-head in column 16

(1) NUMBER OF STRIP 1/20 INCH WIDE PARALLEL TO AXIS A–A	(2) DISTANCE AXIS A–A TO CENTER OF GRAVITY OF STRIP, IN UNITS 1/20 INCH	(3) AREA OF COMPACT BONE IN STRIP IN UNITS 1/20 INCH X 1/20 INCH	(4) STATIC MOMENT OF STRIP. COL. 2 X COL. 3 (1/20 INCH)^3	(5) MOMENT OF INERTIA OF STRIP COL. 2 X COL. 2 X COL. 3 (1/20 INCH)^4	(6) AREA OF SPONGY BONE IN STRIP IN UNITS 1/20 INCH X 1/20 INCH	(7) STATIC MOMENT OF SPONGY BONE IN STRIP. COL. 2 X COL. 6 (1/20 INCH)^3	(8) MOMENT OF INERTIA OF SPONGY BONE IN STRIP. COL. 2 X COL. 2 X COL. 6. (1/20 INCH)^4	(9) STATIC MOMENT OF SPONGY BONE IN TERMS OF COMPACT BONE. 0.187 X COL. 7	(10) TOTAL STATIC MOMENT IN TERMS OF COMPACT BONE. COL. 4 + COL. 9	(11) STATIC MOMENT OF AREA EXTERNAL TO STRIP	(12) THICKNESS OF SHEARING PLANE OF SPONGY BONE AS EQUIVALENT COMPACT BONE. COL. 6 X 0.187	(13) TOTAL THICKNESS OF SHEARING PLANE IN TERMS OF COMPACT BONE. COL. 3 + COL. 12 = t.	(14) $r^2t = \frac{0.06669}{0.424} \times 400 \times$ COL. 13 $= 63.2 \times$ COL. 13	(15) Q = COEFFICIENT OF HORIZONTAL SHEAR = $\frac{\text{COL. 11}}{\text{COL. 14}}$	(16) ACTUAL HORIZONTAL SHEARING UNIT-STRESS AT POINT = COL. 15 X. AVERAGE VERTICAL UNIT-STRESS = COL. 15 X 166. LBS. PER SQ. INCH
0										581.9	3.6	6.6	417	1.395	231
1	0.5	3.0	1.5	0.7	19	10	5	1.9	3.4		3.6	6.6			
2	1.5	3.0	4.5	6.7	19	29	43	5.4	9.9	568.6	3.6	6.6	417	1.365	226
3	2.5	3.0	7.5	18.7	19	47	118	8.8	16.3		3.6	6.6			
4	3.5	3.0	10.5	36.7	18	63	221	12.0	22.5	529.8	3.4	6.4	404	1.310	217
5	4.5	3.0	13.5	60.7	18	81	365	15.3	28.8		3.4	6.4			
6	5.5	3.0	16.5	90.7	17	93	512	17.7	34.2	466.8	3.2	6.2	392	1.190	198
7	6.5	3.0	19.5	127.0	16	103	668	19.6	39.1		3.0	6.0			
8	7.5	3.0	22.5	169.0	16	120	900	22.8	45.3	382.4	3.0	6.0	379	1.035	172
9	8.5	3.5	29.7	253.0	16	136	1,156	25.8	55.5		3.0	6.5			
10	9.5	2.5	23.7	225.0	14	133	1,263	25.3	49.0	277.9	2.7	5.2	328	0.847	141
11	10.5	2.5	26.2	275.0	10	105	1,102	20.0	46.2		1.9	4.4			
12	11.5	3.0	34.5	397.0	9	103	1,184	19.6	54.1	177.6	1.7	4.7	296	0.599	99
13	12.5	3.0	37.5	468.0	7	88	1,100	16.8	54.3		1.3	4.3			
14	13.5	3.0	40.5	546.0	4	54	729	10.3	50.8	72.5	0.8	3.8	240	0.331	55
1	14.5	5.0	72.5	1,051.0					72.5			5.0			
		46.5	359.6	3,725.2	202	1,165	9,366	221.3	581.9						

Lateral to Axis A-A.

Medial to Axis B-B.	0										574.4	3.6	6.6	417	1.378	229
	1	0.5	3.0	1.5	0.7	19	10	5	1.9	3.4		3.6	6.6			
	2	1.5	3.0	4.5	6.7	19	29	43	5.4	9.9	548.8	3.6	6.6	417	1.315	218
	3	2.5	3.0	7.5	18.7	19	47	118	8.8	16.3		3.6	6.6			
	4	3.5	3.0	10.5	36.7	19	66	231	17.3	22.7	509.8	3.6	6.6	417	1.223	203
	5	4.5	3.0	13.5	60.7	18	81	365	15.2	28.7		3.4	6.4			
	6	5.5	3.0	16.5	90.7	18	99	544	18.5	35.0	446.1	3.4	6.4	404	1.103	183
	7	6.5	3.5	22.7	148.0	16	104	676	19.3	42.0		3.0	6.5			
	8	7.5	3.5	26.2	196.5	15	113	848	21.1	47.3	369.1	2.8	6.3	399	0.924	153
	9	8.5	3.5	29.7	252.0	14	119	1,012	22.2	51.9		2.6	6.1			
	10	9.5	4.5	42.8	406.0	12	114	1,083	21.3	64.1	253.1	2.2	6.7	424	0.597	99
	11	10.5	4.0	42.0	441.0	10	105	1,102	19.6	61.6		1.9	5.9			
	12	11.5	3.0	34.5	396.0	9	103	1,184	19.3	53.8	137.7	1.7	4.7	296	0.464	77
	13	12.5	4.5	56.2	703.0	6	75	937	14.0	70.2		1.1	5.6			
	14	13.5	5.0	67.5	910.0					67.5			5.0			
			49.5	375.6	3,666.7	194	1,065	8,148	203.9	574.4						
			46.5	359.6	3,725.2	202	1,165	9,366	221.3	581.9						
Totals			96.0	735.2	7,391.9	396	2,230	17,514	425.2	1,156.3						

Total area as compact bone $\begin{cases} = 96.0 + (0.187 \times 396.0) = 170. \\ = 170.0 \div 400 = 0.424 \text{ square inches.} \end{cases}$

Total moment of inertia in terms of compact bone... $\begin{cases} = 7391.9 + (0.187 \times 17514.0) = 7391.9 + 3275. \\ = 10667.0 \div 160{,}000 = 0.06669 \text{ inches.}^4 \end{cases}$

moment of inertia of the entire section is the sum of the moment of inertia of the compact bone and the effective moment of inertia of the cancellated bone.

5. Analysis of sections. The computations in complete detail are given in tabular form, in table 4, for the effective moment of inertia of section 8, which illustrates the methods employed throughout for the calculation of the moment of inertia. Figure 15 shows section 8 with the system of lines drawn at intervals of $\frac{1}{20}$ inch and parallel to the two principal axes of the section. This figure forms the basis for the computations of table 4.

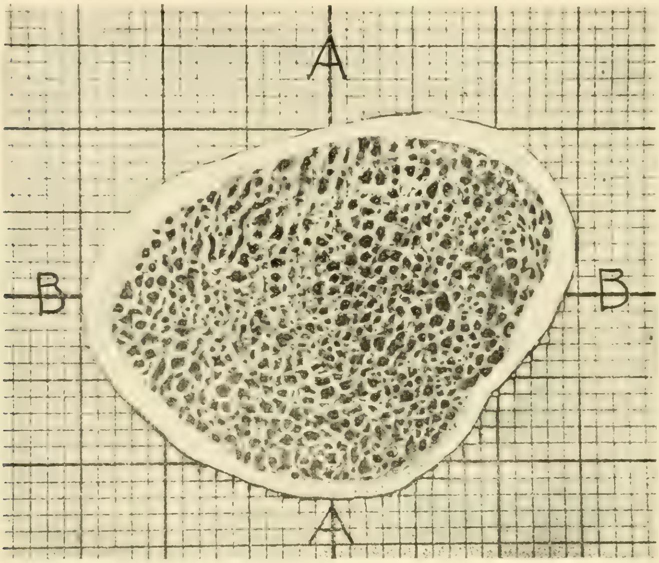


Fig. 15 Transverse section 8 of normal femur. From the same subject as figure 14. Enlarged figure with ruling of $\frac{1}{20}$ inch squares, parallel to axis A–A, to accompany table 4 which gives the complete calculations for the mechanical properties of this section about axis A–A. See 'Analysis of Sections,' Part III. Section 8 is shown natural size in Plate 1.

In a similar manner the moments of inertia of the other sections have been calculated and reduced to terms of moment of inertia of compact bone, which renders comparable the bending strengths of all the sections.

Table 5 gives the area of spongy and of compact bone, the equivalent area of compact bone, the moment of inertia, and other data for each of the transverse sections analyzed. These data are called the properties of the sections. From the information given in this table the effects produced at every section by the external load may be computed. The moments of inertia are computed about both principal axes, *A–A* and *B–B*, for completeness; but only those about axis *A–A* are required to compute the effects of the load on the femur-head.

6. Bending strength. The bending strength of the femur at each section is measured by the section modulus of the section. The general formula for the determination of the section modulus has been given in Part II (par. *41*). The values of the section moduli are given in table 5, columns 14 and 21 (p. 235).

The bending strength of the femur about any other plane than that of *A–A* may be determined, since the moment of inertia is computed about the two principal axes *A–A* and *B–B* which are at right angles and these are principal moments of inertia.

7. Torsional strength. The resistance to twisting action is known as torsional strength, and it is measured by the sum of the moments of inertia about the two principal axes, *A–A* and *B–B* in the sections shown in plates 1–5.

Practically all muscles, being attached to the surface of the bones, must produce twisting to a certain degree when exerting a pull upon any bone. The extent of the twisting or torsional stress depends upon the amount of the pull and the angle between the direction of the pull and that of the longitudinal axis of the bone. Such stresses combine with the maximum tensile and compressive stresses in the femur (and other weight-bearing bones) to produce still greater stresses than those due solely to the weight borne by the bone.

A further consideration of the manner of attachment of muscle to bone makes clear that in addition to the torsion in the bone produced by a muscle pull there is a tensile force acting in a direction opposite to the pull and a compression in the bone, in the fibers, between the attachment of the muscle and the joint about which the force is exerted.

Effects of external load

1. Shearing and axial loads. Referring to figure 14, the load transmitted to the head of the femur is distributed over the upper surface of the head between points G and H, as shown in longitudinal section, and practically all of the head of the femur above the point E is in bearing with the acetabulum, receiving a load that is practically uniformly distributed.

As the joint surface is smooth, the forces applied to the head of the femur are everywhere perpendicular to the surface of the femur-head. All the forces at the joint may, for simplicity, be considered as acting in the line AB, in the center of pressure of the femur-head, and acting as a concentrated load W, which is the sum of all the vertical components of the forces acting at the joint. The longitudinal axis of the femur, $EDCB$, is nowhere

TABLE 5

Mechanical properties of the normal femur

Column 1 indicates the section, the position of which is indicated by the corresponding number in figures 14, 17, 18, 19, 19a, 20 and 27

Column 2 gives the area of the entire section in square inches

Column 3 gives the area of the section occupied by compact bone

Column 4 gives the area of the section occupied by spongy bone

Column 5 gives the density of the spongy bone of the section as compared with compact bone

Column 6 gives the area of the spongy bone of the section in terms of compact bone

Column 7 gives the total area of the section in terms of compact bone

Columns 8 and 9 give the distance from the axis A–A to the extreme tension and compression fibers, respectively

Columns 10 to 13 give the moments of inertia (in biquadratic inches) for the compact bone, the spongy bone, the equivalent of the spongy bone and the combined or total moment of inertia of the section, respectively, all except column 11 being expressed in terms of compact bone

Column 14 gives the value of the section modulus, which is the strength ratio of the section with respect to axis A–A. This is obtained by dividing the moment of inertia about axis A–A by the distance from this axis to the extreme fiber of the section

Columns 15 to 18 give the moments of inertia about axis B–B as indicated, all except column 16 being expressed in terms of compact bone

Columns 19 and 20 give the distance from the axis B–B to the extreme anterior and posterior fibers, respectively

Column 21 gives the value of the section modulus (strength ratio) with respect to axis B–B. This is obtained by dividing the moment of inertia of the section about this axis by the distance from this axis to the extreme fiber

TABLE 5

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Section Number	Areas			Ratio of Compactness of Spongy Bone.	Equivalent Area of Spongy Bone as Compact Bone, Cols 4 x 5.	Area of Section as Compact Bone. Total. Col 3 + Col. 6	Distance from Axis A-A to Extreme Tension Fibre	Distance from Axis A-A to Extreme Compression Fibre.	Moment of Inertia about Axis A-A				Section Modulus Col. 13 ÷ larger of Cols 8 and 9. Axis A-A	Moment of Inertia about Axis B-B				Distance from Axis B-B to Extreme Anterior Fibre	Distance from Axis B-B to Extreme Posterior Fibre	Section Modulus Col. 18 ÷ larger of Cols. 19 and 20. Axis B-B.
	Gross Area.	Compact Bone	Spongy Bone						Compact Bone	Spongy Bone.	Equiv of Spongy Bone Cols 11 x 5	Combined Moment Col. 10 + Col. 12		Compact Bone	Spongy Bone	Equiv of Spongy Bone Col. 16 × Col 5	Combined Moment Col 15 + Col 17			
	Sq In	Sq In	Sq In		Sq Ins	Sq Ins	Inches	Inches	(Inches)^4	(Inches)^4	(Inches)^4	(Inches)^4	Inches^3	(Inches)^4	(Inches)^4	(Inches)^4	(Inches)^4	Inches	Inches	Inches^3
2	137	—	137	470	0643	0643	066	0.66	—	.4585	2155	.2155	.326	—	4585	2155	2155	066	066	.320
4	269	—	269	304	816	816	092	0.92	—	5750	1748	1748	.190	—	5750	1748	1748	92	.92	190
6	187	—	187	282	527	527	0.85	0.85	—	3310	0933	0933	.110	—	2240	0631	0634	70	70	091
8	124	237	1.00	.187	.187	424	074	0.74	.0462	1097	0205	0667	.090	.0320	0645	0121	.0441	.55	58	076
10	233	459	188	.092	173	632	103	1.26	.2480	.6320	0582	3062	245	0875	.2045	0188	1063	75	90	118
12	254	642	190	147	.279	921	114	112	.3722	5168	0759	4481	.392	.1407	2519	0373	1780	74	76	234
14	240	600	180	038	.069	.069	112	0.92	.2595	.4185	0159	2754	.246	.1097	2421	0092	1189	.65	79	151
16	198	629	135	088	119	748	096	0.64	.1695	2325	0205	1900	198	1240	1755	.0155	1395	65	84	160
18	134	637	071	102	.072	709	075	0.57	.1217	0478	0049	1266	.169	.0853	0443	.0045	.0898	63	56	.143
20	107	.681	039	055	.022	703	0.58	0.64	.0922	0098	00054	.0927	145	0700	.0078	.0004	0710	.55	60	.118
24	103	740	029	086	025	765	057	0.56	.0784	0034	0003	0787	.138	.0756	0111	0010	.0706	.58	58	132
28	1.06	758	030	125	.037	795	052	0.59	.0715	.0057	0007	0722	.123	0844	0090	0011	0855	56	61	140
32	104	822	—	—	—	822	052	0.53	.0697	—	—	0697	132	0950	—		0956	.54	69	139
36	103	806	·	—	—	806	050	0.58	.0625	—	—	0625	.108	1040	-	-	1040	53	75	139
40	105	775	—	—	—	775	0.50	0.59	.0665	—	—	0665	.113	1048	—	—	1048	56	.76	.138
44	105	771	—	—	—	.771	0.50	0.62	.0686	—	—	0686	.111	1032	—	—	1032	.60	78	.132
48	105	.760	—	—	—	.760	0.54	0.60	.0702	—	—	0702	117	.1056			1056	59	76	139
52	105	679	—	—	—	679	055	0.60	.0672	—	—	.0672	112	0877	-		0877	59	68	129
56	115	687	030	.107	.032	.719	059	0.65	.0863	.0120	.0013	.0876	133	0907	0100	0010	.0977	62	.60	.140
60	131	652	0.35	.070	047	.699	0.63	0.73	.1088	0160	0011	.1099	149	1012	0143	0010	1022	.65	63	156
64	151	527	074	114	080	607	0.71	0.78	.1350	0615	0010	.1420	173	0940	0.140	0050	.0990	66	60	142
66	1.70	.528	117	096	0.112	.640	072	0.89	.1432	1588	0152	.1584	.178	.0978	1039	.0100	1078	79	.54	136
68	196	485	147	.093	0137	622	089	0.87	.1725	2711	.0116	1841	206	1042	1259	.0054	1096	80	.58	137
70	262	.470	215	120	0258	728	105	1.02	.2510	.6004	0720	3230	.307	1312	3015	0362	1674	1.00	52	167
72	403	.345	368	.154	0.566	911	149	1.26	.2810	1.8542	.2854	.5664	380	1725	8581	1310	3035	1.28	76	231
75	594	—	594	.253	1.500	1500	1.72	1.50	—	40161	1.0135	1.0135	590	—	32939	.8318	.8318	1.70	1.02	489

parallel to the line of action of the load, *W*, which acts in the direction *AB*.

Hence, at successive points along the axis of the femur the load, *W*, is resolved into two components parallel and perpendicular, respectively, to the axis *EDCB*. The component parallel to the axis at any given point is the axial load and the component at right angles to this force is the vertical shear at the given point. Vertical shear is therefore the component of the load which is perpendicular to the axis of the bone.

In figure 14 the lines marking the successive sections are indicated by the radial lines *1*, *2*, *4*, *6*, etc., all of which except *1* and *2*, intersect at a common point *F*. These lines have been discussed in greater detail in a preceding section, under the heading, "Analysis of serial transverse sections" (p. 227).

2. Resolution of load. A. Axial load and vertical shear. In order to determine graphically the amount of the axial and of the vertical shearing force at successive sections of the femur under the assumed load, pass a line *FJ* in figure 14, through *F* and parallel to *AB*. The force *W*, is laid off to scale as *FJ*, and for simplicity it will be assumed to be 100 pounds. By this means percentages of the load may be readily computed, and subsequently the stresses finally determined, may be calculated for any other load on the femur by simple proportion. The accuracy of the calculations is not affected by this procedure and the labor of computation is much reduced.

From the point *J* draw the lines *JK*, *JL*, *JM*, *JN*, *JO*, *JP*, *JQ*, and *JR*, perpendicular respectively to the radial lines *F4*, *F6*, *F8*, *F10*, *F12*, *F14*, *F16* and *F17*. Then the line *JK* represents to scale the direction and amount of the component of *W* which acts at right angles to *F4*, or the axial load acting on section *4* of the femur. Likewise, *FK* represents the component of *W* which acts in a direction perpendicular to the axis of the femur at section 4, and therefore represents the vertical shearing force at that section.

In the same manner the components of *W* which form the axial force and the vertical shearing force at successive sections are indicated in figure 14. The axial forces are indicated at sections 6, 8, 10, etc. to 17 by the lines *JL*, *JM*, *JN*, *JO*, *JP*, *JQ* and *JR*, respectively. The vertical shearing forces at the same successive sections are represented in the same figure by the lines *FL*, *FM*, *FN*, *FO*, *FP*, *FQ*, and *FR*, respectively. After section 17 is passed the character of the vertical shearing force is reversed, but its amount is so small as to be negligible.

The amounts of these axial and shearing forces are shown in figure 16, in diagrammatic form for the whole length of the femur. Table 6 (cols. 2 and 4) gives the numerical values of these axial and vertical shearing forces.

3. Bending moment. In addition to the axial loads and the vertical shears, the load on the femur-head produces a tendency to cause bending in the femur. In accordance with the definition previously given (Part II, *26*), the amount of the bending moment is the product of the force acting on the section by the perpendicular distance from the point to the line of action of the force. The line of action of the force is *AB* (fig. 14) and its amount *W*, is 100 pounds. which remains a constant for the whole length of the femur. Hence, the bending moment at successive points will be proportional to the perpendicular distance from the given point (on the neutral axis) to *AB*. The numerical values of the bending moment at successive sections are given in table 6 (col. 3), and in figure 16 these values are shown in diagrammatic form (p. 240).

It will be noted that the bending moment rapidly increases in the head and neck of the femur to reach a maximum between sections 16 and 18. Beyond the latter section the amount of the bending moment diminishes almost uniformly to zero just below section 75.

4. Vertical and horizontal shear. As a fundamental principle in mechanics, it may be stated that at any point in any beam the horizontal shearing force is equal to the vertical shearing force at the same point. The intensity of the horizontal shearing force, in any transverse section, varies from a minimum at the fibers most distant from the neutral axis to a maximum in the neutral plane (Part II, *50*, *51*), the variations depending upon the shape of the cross section. Hence it will be necessary to compute the intensity of the horizontal shearing force at a number of points in each section of the head and neck of the femur where the shearing forces produce their principal effects. In each of these transverse sections the coefficients, Q, of the intensity of the horizontal shearing stress were computed at intervals of $\frac{1}{10}$ inch from the neutral surface of the femur. The horizontal shearing unit-stress at these points is found by multiplying the average vertical shearing unit-stress for the section by the coefficient Q, for the given point.

The method of computing the intensity of the horizontal shear is shown in table 4, which gives the complete calculations in tabular form for transverse section 8. In similar manner, the intensity of the horizontal shearing forces have been computed for the other even-numbered sections from 4 to 18 inclusive.

Having found the horizontal shearing unit-stresses for the various points in each section, the vertical shearing unit-stresses are also known as they are equal in amount at corresponding points, and differ only in being directed at right angles to the horizontal shears.

5. The unit-stresses due to the external load. A. Axial unit-stress. The axial unit-stress is the axial load at the section divided by the net area of the transverse section. In the sections containing spongy bone the area is expressed in terms of the equivalent area of compact bone. The unit-stresses have been computed for each section by dividing the axial load by the

TABLE 6

Analysis of stresses in the normal femur produced by a load of 100 pounds: by walking and by running

In this table there is given in condensed form the analysis of the stresses produced in successive sections of the normal femur, by a load of 100 pounds applied to the head of the femur in the same direction as the body weight is transmitted. From the data tabulated the maximum unit-stresses (lbs. per sq. in.) at the various sections is computed for the load of 100 pounds. Then by simple proportion, in accordance with the principles of mechanics the maximum stresses at these sections are calculated for a load of 160 pounds and for a load of 320 pounds on the femur, being the approximate load carried by the femur in walking and in running, respectively. The numbers of the sections given in column 1 correspond to the numbers of the sections in figure 18 and all other diagrams and tables similarly numbered

TABLE 6

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	100 Pounds Load on Femur-Head (Assumed Load).												160 Lbs. on Femur. DUE TO WALKING. Max. Unit-Stresses			320 Lbs on Femur DUE TO RUNNING Max. Unit-Stresses.			Factors of Safety for RUNNING	
Section Number	Axial Load on Section	Bending Moment about Axis AA	Total Vertical Shear on Section	Unit-Stresses Due To				Q	Maximum Unit-Stresses											
				Axial Load Uniform over Section.	Bend'g Moment Maximum Tensile Stress. (Lateral)	Bend'g Moment Maximum Compressive Stress (Medial)	Vertical Shear. Averge over Section	Coefficient of Horiz'l Shear on Neutral Plane.	Tensile Extreme Lateral Fibre.	Compressive. Extreme Medial Fibre.	Horizontal Shear. On Neutral Plane Col. 8 x Col. 9.	Vertical Shear	Tensile Extreme Lateral Fibre.	Compressive. Extreme Medial Fibre.	Horizontal and Vertical Shear. on Neutral Plane.	Tensile Extreme Lateral Fibre	Compressive. Extreme Medial Fibre	Horizontal and Vertical Shear. on Neutral Plane	Tensile 17,700 / Col 17	Compressive 24,000 / Col 18
	Pounds	Inch-Pounds	Pounds.	Pounds Per Sq In					Pounds Per Sq In.				Pounds Per Sq In.			Pounds Per Sq In.				
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	400	29	+91.8	49.	152	152.	+112.5	1.33	103	201	+163	+163	165	322.	260	330	644	520	5360	3725
6	66.0	71.	82.5	125.	646.	646.	+156.5	1.33	521	771.	+208.	+208.	833.	1232.	332	1666	2464	664.	1062	974
8	71.0	103.	70.3	168.	1142.	1142.	+166.0	1.39	974.	1310.	+231.	+231.	1560.	2098.	369.	3117.	4192.	738	568	5.71
10	82.5	132.	55.8	130.	445.	543.	+88.2	1.42	315.	673.	+125.	+125.	504.	1078.	200.	1008.	2156.	400	1756	1115
12	92.0	149.	39.5	100.	379.	373.	+42.8	1.40	279.	473.	+ 60.	+ 60.	447.	757.	96.	894.	1514.	192.	19.80	1585
14	97.0	161.	21.8	145.	654.	538.	+32.5	1.40	509.	683.	+ 45.	+ 45.	815.	1095.	72.	1630	2190.	144	1088	1096
16	99.0	162.	4.0	132.	818.	548.	+ 5.3	1.37	686.	680.	+ 7.	+ 7.	1098.	1090.	11.	2196.	2180.	22.	8.06	1100
18	100.0	155.	− 5.7	141.	917.	696.	− 8.1	1.33	776.	837.	− 11.	− 11.	1240.	1340.	−18.	2480.	2680.	− 36.	7.14	8.96
20	100.	152.	−5.7	142.	951.	1049.	−8.1	1.33	809.	1191.	−11.	−11.	1297.	1906.	−18.	2594.	3812.	−36	6.82	630
24		145.		131.	1052.	1029.			921.	1160.			1473.	1854.		2946.	3708.		6.01	6.47
28		138.		126.	993.	1127.			867	1253.			1386.	2005.		2772	4010		6.38	598
32		128.		121.	954.	972.			833.	1093.			1334.	1750.		2668	3500.		663	685
36		119.		124.	952.	1103.			828.	1227.			1326.	1963.		2652	3926		668	612
40		106		129.	798.	939.			669.	1068.			1070.	1710.		2140	3420		827	7.02
44		94.		130.	684.	850.			554.	980.			886.	1568.		1772	3136.		998	768
48		82.		132.	629.	702.			497.	834.			795.	1337.		1590.	2674.		1114	8.96
52		75.		147.	613.	669.			466.	816.			745.	1305.		1490	2610		1189	9.19
56		57.		139.	390.	420.			251.	559.			402.	894.		804	1788		2200	1343
60		44.		143.	255.	203.			112.	346.			179.	553.		358.	1106		1950	2174
64		33.		164.	173.	191.			9.	355.			14.	568.		28	1136		—	2112
66		27		156.	123.	151.			Compression 33.	307.			Compression 53.	491.		Compression 106	982		—	2440
68		20.		183.	97.	106.			86.	289.			137.	463.		274.	926.		—	2590
70		14		137.	46.	44.			91.	181.			146.	289.		292.	578		—	4150
72		8.		110.	34.	18.			76.	128.			121.	205.		242	410		—	5850
75		0.		65.	0.	0.			65.	65.			104.	104.		208	208		—	11950

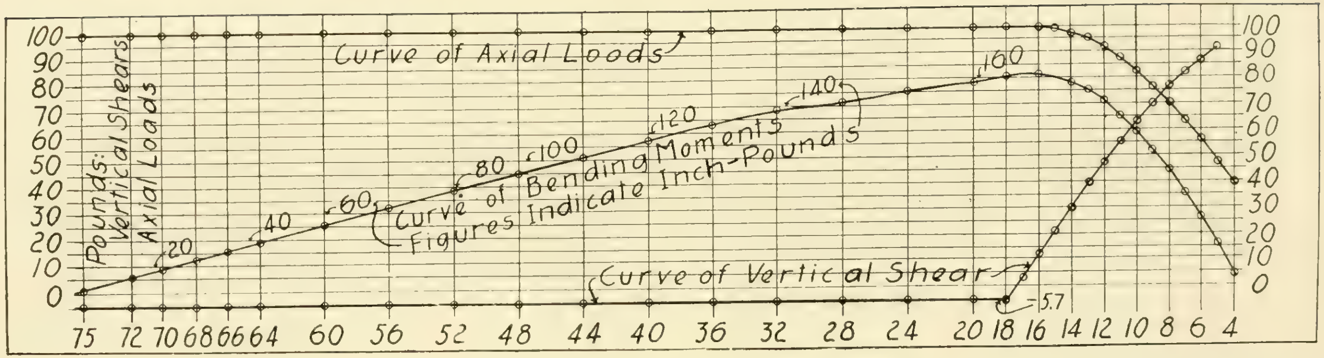


Fig. 16 Diagram of axial loads, bending moments and vertical shears for 100-pound load on the normal femur whose force diagram is given in figure 14. The section numbers at the bott om of this diagram correspond to the numbered sections of figure 14. See discussions under 'Effects of external load.'

equivalent area of compact bone. These results are shown in diagrammatic form for the entire length of the femur in figure 17. In figure 18*a* the axial unit-stress is shown to scale for section 8, due to the load of 100 pounds on the femur-head. Table 6, Column 2 gives the numerical value of the axial load, and Column 5 the corresponding axial unit-stresses for the whole femur.

B. Unit-stresses due to bending moment. As has been explained in Part II (*26–41*) in the discussion of beams, the effect of bending moment is to cause tensile stresses on one side of the neutral axis and compressive stresses on the opposite side. The amount of this unit-stress depends upon the distance of the fibers considered from the neutral axis. These stresses are proportional to the distance from the neutral plane, and are zero at this plane. Hence the maximum unit-stresses due to bending moment will be in the outermost fibers of any given section.

By the application of the formula (Part II, *39*), $s = Mc/I$, the value of the maximum unit-stress due to bending moment can be readily computed for the sections analyzed. The value of M, the bending moment, for successive sections is given graphically in figure 16 and numerically in table 6. The value of c, the distance from the neutral plane to the outermost fiber, and of I the moment of inertia of the cross section are given in table 5, table of properties of femur sections. In figure 18*a* the stresses due to the bending moment are shown for section 8. A similar diagram could be constructed for all the other sections analyzed.

In figure 17 curves 3 and 4 show graphically the values of the maximum unit-stresses, tensile and compressive, due to the bending moments produced by a load of 100 pounds on the femur.

C. Horizontal and vertical shearing unit-stresses. The average vertical shearing unit-stress at each section is derived by dividing the vertical shear at the section by the net area of compact bone of the section. Then the actual horizontal shearing unit-stress is computed by multiplying this average shear-

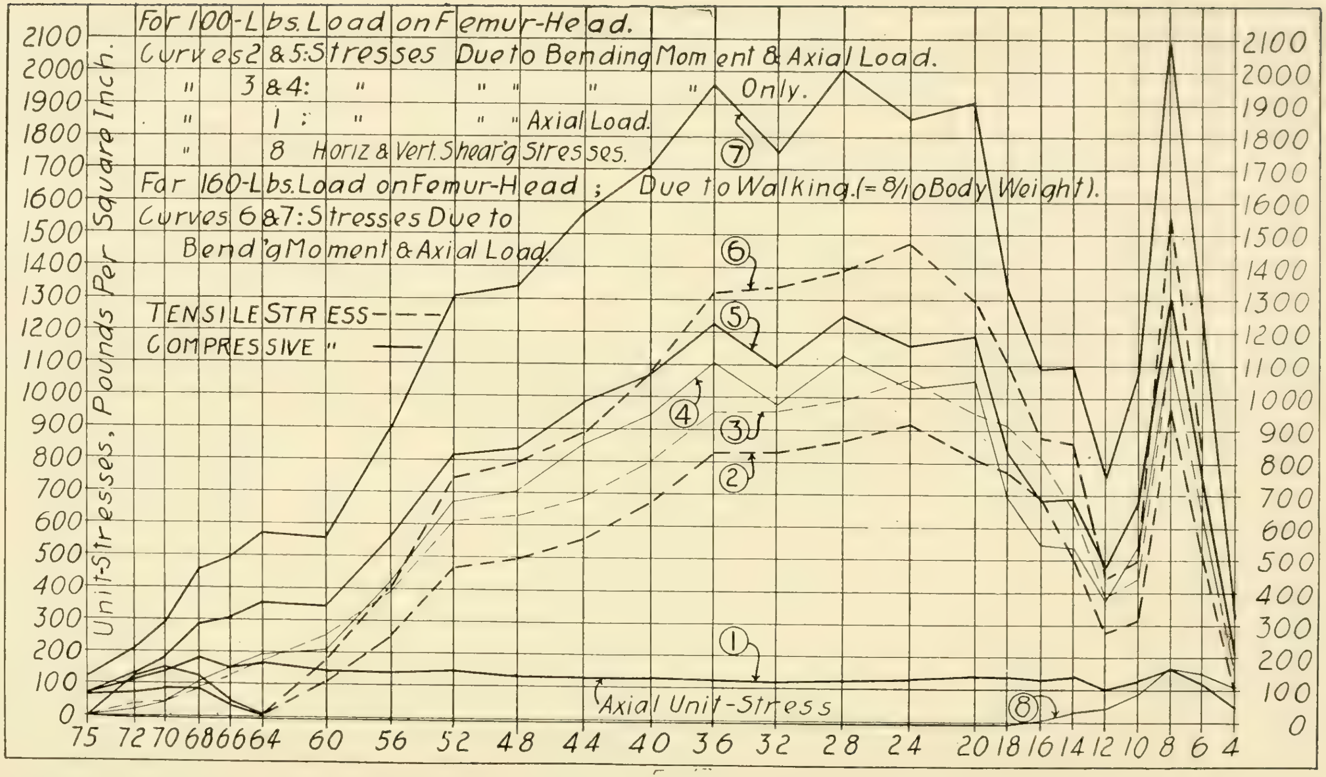


Fig. 17 Diagram of maximum unit-stresses in the normal femur, for loads as shown. Numbers at bottom of diagram correspond to the numbered sections shown in figure 14. Stresses are given in detail for a load of 100 pounds on the femur-head. Only the maximum tensile and compressive unit-stresses are given for the load due to walking, taken at 0.8 of the body weight of the subject from whom this femur was taken.

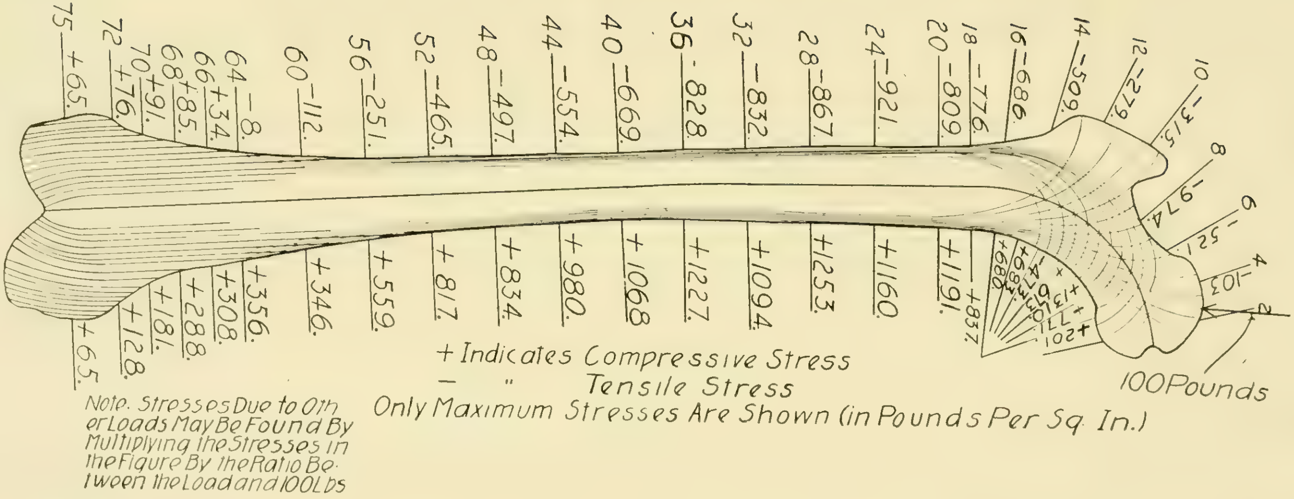


Fig. 18 Diagram of the computed lines of maximum stress in the normal femur. The section numbers correspond to the sections indicated in figure 14 and succeeding figures. The amounts of the maximum tensile and compressive stress at the various sections are given for a load of 100 pounds on the femur-head. For the standing position ('at attention') these stresses are multiplied by 0.6. For walking these stresses are to be multiplied by 1.6, and for running these stresses are to be multiplied by 3.2.

ing unit-stress by the coefficient Q, previously determined for each given point in each section. The details of such computations are given for section 8 in table 4 (p. 230); the same methods were employed for all the other sections analysed.

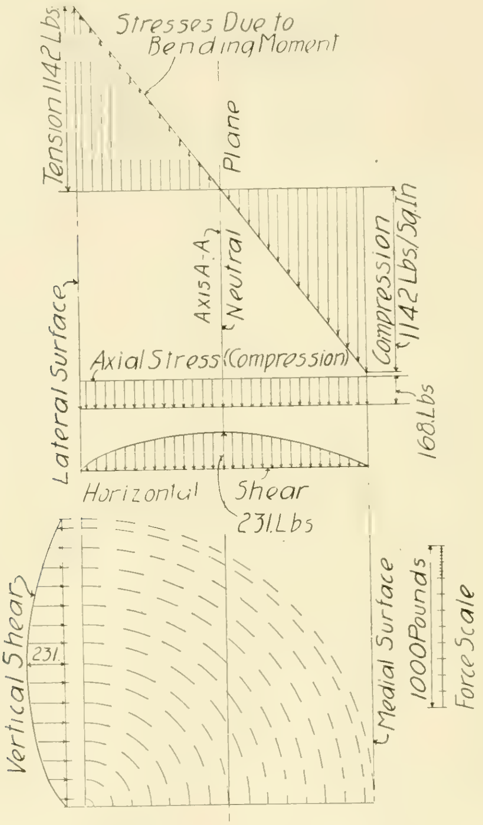


Fig. 18 *a* Diagram of the stresses in transverse section 8, based on a load of 100 pounds on the femur-head. The amounts and lines of action of the stresses in every part of this section are shown graphically to scale. There are four kinds of stress in action at every point in the section: compression uniformly distributed over the cross section; tension or compression due to the bending moment, such stress varies uniformly from zero at the neutral plane to a maximum at the extreme outer fibers of the section, as shown; horizontal shear and vertical shear, both of which are equal in amount at any given point, and which vary from a maximum at the neutral axis to a minimum of zero at the fibers most distant from the neutral axis, the variations depending upon the shape and consistency of the section.

In the calculations for these coefficients their values were computed in each section at intervals of $\frac{1}{10}$ inch from the neutral plane.

In figure 18*a* is shown to scale the diagram of the intensity of the horizontal and vertical shearing unit-stresses for section 8. It will be seen that the horizontal and vertical shearing stresses are equal but are at right angles to each other as has been explained in Part II, (*50–51*).

6. *Maximum unit-stresses in the femur.* The axial unit-stress in the femur is throughout a compressive stress. Hence it combines with the compressive stress due to the bending moment on the medial side of the femur, to produce maximum compressive unit-stresses in the fibers most distant from the neutral axis.

The axial (compressive) unit-stress on the tensile side of the femur balances or offsets an equal amount of tensile unit-stress due to bending moment, thus decreasing the amount of the tensile unit-stress. The true maximum tensile unit-stress is therefore the difference between the intensity of the maximum tensile unit-stress due to the bending moment and the intensity of the axial compressive unit-stress. In figure 18*a* the axial and bending moment stresses are shown to scale for section 8, and the effect of the axial stress is seen in increasing the unit-stress at every point on the medial side and decreasing the tensile unit-stress at every point on the lateral side.

The amounts of these maximum unit-stresses at various points along the femur are shown numerically in table 6, diagrammatically in figure 17 and the amounts and positions graphically in figure 18.

In figure 17 is shown a series of curves representing the maximum unit-stresses at every section due to the assumed load of 100 pounds on the femur-head. Curve 1 gives the intensity of the unit-stress due to the axial load. Curve 3 gives the amount of the maximum tensile unit-stress due to the bending moment at the various sections, and curve 4 gives the amount of the corresponding maximum compressive unit-stresses due to the bending moment. Curve 2 gives the maximum amount of the tensile unit-stress at the various sections due to the combined action of the axial load and the bending moment, while curve 5 gives the corresponding maximum compressive unit-stresses due to the combined action of the axial load and the bending moment.

In this figure curves 7 and 6 show the intensity of the maximum compressive and tensile unit-stresses along the femur due to the combined action of the axial load and the bending moment, for a load of 160 pounds (= 8/10 of body weight) on the femur-head, produced in walking by the subject who weighed 200 pounds.

7. The lines of maximum internal stress. In Part II, *53*, the formulas for computing the magnitudes and directions of the maximum and minimum internal stresses were given. The unit-stresses due to axial load, bending moment and horizontal and vertical shear having been determined for the various sections as described above, the formulas mentioned can now be applied to compute the directions and magnitudes of the maximum and minimum internal unit-stresses throughout the femur.

Distal to section 16 the shearing stresses are quite small (see curve 8, figure 17, giving maximum intensity of horizontal and vertical shearing unit-stresses), and produce no appreciable effect upon the position of the lines of internal stress. For this reason the lines of maximum internal stress become parallel to the longitudinal axis of the femur below this section.

In the head and neck of the femur the stresses due to bending moment and vertical and horizontal shear combine to produce maximum and minimum internal stresses at every point in this region. These maximum and minimum stresses act everywhere in directions perpendicular to each other. Where the maximum tensile stress occurs there is also a minimum compressive stress acting at right angles to it: and conversely, where the maximum compressive stress occurs there is a minimum tensile stress at right angles to it.

As there is an infinite number of points in each transverse section subject to the action of these forces, which vary in intensity at successive points, there is an infinite number of resultant lines of maximum tensile and compressive stress passing through each transverse section of the femur. As it is desired to know the paths of only a few of these resultant lines of stress,

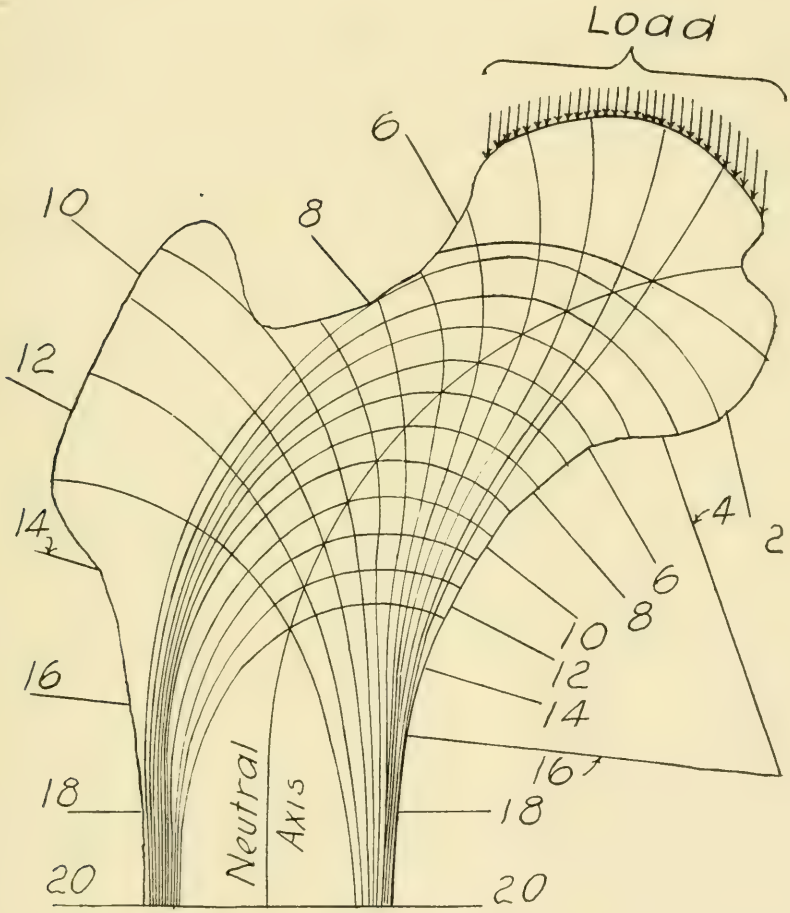


Fig. 19 Diagram of the lines of stress in the upper femur, based upon the mathematical analysis of the right femur. These result from the combination of the different kinds of stress at each point in the femur, which figure 18 *a* shows in detail for section 8.

they may be closely approximated by calculating the amounts and directions of these maximum stresses at a number of points in each of the sections comprising the upper femur.

The directions and amounts of these maximum and minimum internal unit-stresses have been computed in each section at intervals of $\frac{1}{10}$ inch from the neutral axis. In figure 18 the paths of these maximum and minimum stresses are plotted to show their relation to the whole femur. Figure 19 is drawn to full scale to show the position of these paths of resultant stress in the upper femur. In both figures it will be noted that these lines have the following characteristics:

1. All intersections of lines from opposite sides are at right angles.
2. The intersection of all lines with the neutral axis is at an angle of 45 degrees.
3. The lines are in two systems: one, compressive rising on the lateral or outer side of the femur perpendicular to the surface of the femur and crossing the neutral axis and gradually fusing in the medial portion of the shaft; the other, tensile, rising on the medial side of the femur perpendicular to the surface and crossing the neutral axis and fusing gradually below the greater trochanter in the lateral portion of the shaft.

In figure 19 *a* is shown approximately the intensity of the maximum and minimum tensile and compressive stresses calculated for a load of 100 pounds on the femur head. It will be noted that the minimum compressive stresses in all the lines of compression are lateral to the neutral axis, and the maximum compressive stresses are medial to the neutral axis. The minimum tensile stresses in any line of tensile stress are medial to the neutral axis and the maximum stresses are lateral to the neutral axis. The maximum stresses in either system are, in general, found as the direction of the line of stress becomes parallel to the neutral axis. As these lines leave the neutral axis and gradually approach a direction perpendicular to the neutral axis they approach a value of zero.

PART IV. THE NORMAL INNER ARCHITECTURE OF THE FEMUR

For clearness of description of the inner architecture of the human femur, it may be divided into three parts: the upper femur, shaft and distal portion. The former includes the head and neck and extends to the lower limit of the lesser trochanter.

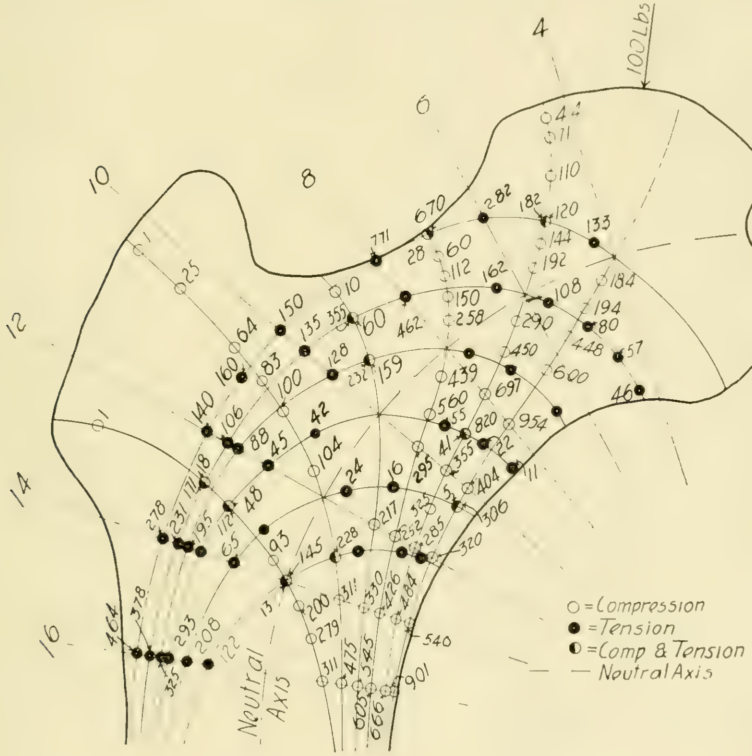


Fig. 19 *a* Intensity of the maximum tensile and compressive stresses in the femur-head. Computed for the load of 100 pounds on the right femur. Corresponds to the upper part of figure 18.

The shaft includes all that portion of the femur which lies between the lesser trochanter and the distal portion. The distal portion is taken as the lower-most six inches of the femur and includes all that part of the bone in which the shaft gradually increases in size to form the articular surface of the lower end

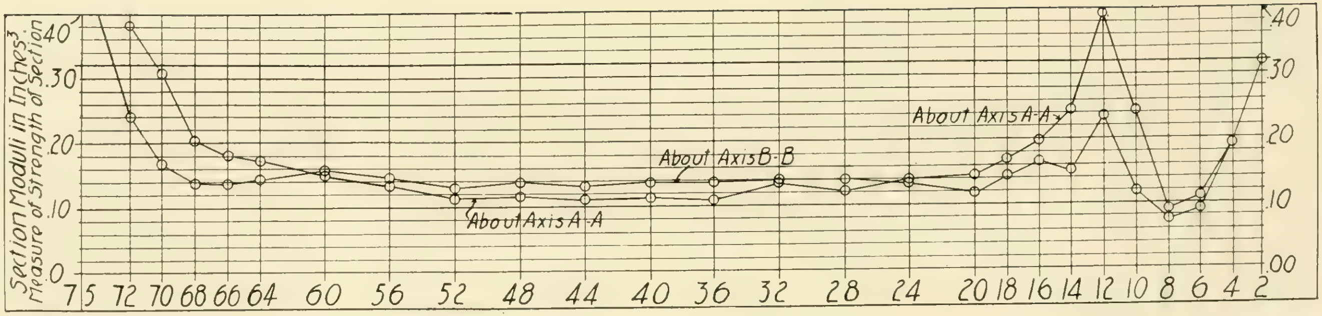


Fig. 20 Diagram of the relative strengths of the femur about its two principal axes. Values of the section modulus are plotted to scale for each axis.

of the femur. These divisions differ very markedly in structure, and the principles of mechanics involved in each require separate consideration.

The inner architecture of the upper femur

1. *Serial frontal longitudinal sections.* The sections are cut in planes parallel to the plane passing through the longitudinal axis of the shaft and the head and neck of the femur, which is directed medially and about 12 degrees anterior to the true frontal plane of the body.

Figure 21 and figure 28 represent such a section cut through the central part of the femur and very close to the longitudinal axis of this bone. Comparison of this figure with those showing the parallel sections adjacent to it shows a very close similarity of the inner architecture of all these sections. For this reason the detailed description given for the mid-section shown in figure 21 will also apply to the others.

Referring to this figure, it is seen that the spongy bone of the upper femur is composed of two distinct systems of trabeculae arranged in curved paths: one, which has its origin in the medial (inner) side of the shaft and curving upward in a fan-like radiation to the opposite side of the bone; the other, having its origin in the lateral (outer) portion of the shaft and arching upward and medially to end in the upper surface of the greater trochanter, neck and head. These two systems intersect each other at right angles. The spaces between these intersecting systems of filaments are of variable size and shape, depending upon their position. In the head of the femur these spaces are very small and in the lower portion of the section they are of much greater size. The two systems of trabeculae will now be discussed in detail.

A. Medial (compressive) system of trabeculae. As the compact bone of the medial (inner) part of the shaft nears the head of the femur it gradually becomes thinner and finally reaches the articular surface of the head as a very thin layer. From a point at about the lower level of the lesser trochanter, 2½ to 3 inches from the lower limit of the articular surface of the head, the trabeculae branch off from the shaft in smooth curves, spreading radially to cross to the opposite side in two well-defined groups: a lower, or secondary group, and an upper, or principal group. The former consists of the thin light trabeculae which cross over from the medial part of the shaft to end in the opposite side in the region of the greater trochanter and in the

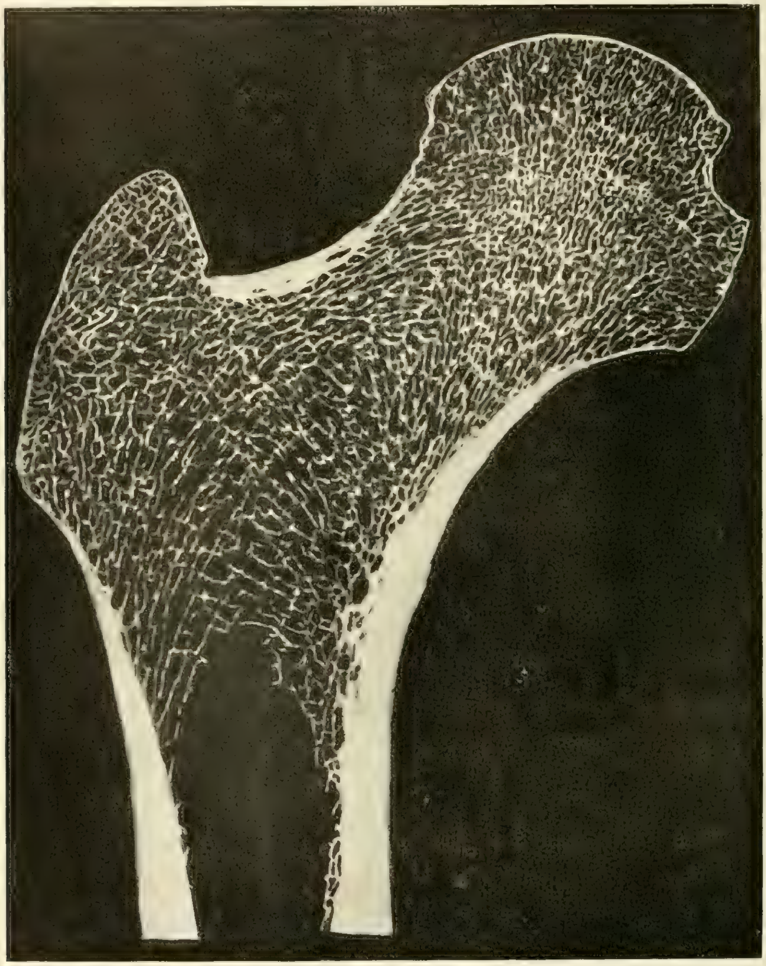


Fig. 21 Frontal longitudinal mid-section of upper left femur. Companion to the right femur analyzed in Part III. The same femur is shown in figs. 24 and 25. (Natural size.)

neck of the femur. The principal group consists of much heavier trabeculae, all of which cross from the medial part of the shaft to end in the upper articular surface of the head.

a. The secondary compressive group. This group of trabeculae leaves the inner border of the shaft beginning at about the level of the lesser trochanter, and for a distance of almost 2 inches along the curving shaft, with which the separate trabeculae make an angle of about 45 degrees. They curve outwardly and upwardly to cross in radiating, smooth curves to the opposite side. The lower filaments end in the region of the great trochanter; the adjacent filaments above these pursue a more nearly vertical course and end in the upper portion of the neck of the femur.

The trabeculae of this group are thin and with wide spaces between them. As they traverse the space between the medial and lateral surfaces of the bone they cross at right angles the system of curved trabeculae which rises from the lateral (outer) portion of the shaft.

It may not be amiss here to call attention to what may appear to be a trivial detail: the question as to whether these intersections are always at 90 degrees, and if some of the angles are not acute or obtuse angles. It has been strongly denied by various authors (Albert '00) that these intersections of the trabeculae of the head of the femur are at angles of 90 degrees. For the purpose of illustrating how deceptive right-angle intersections of curves may appear, figure 22 shows the intersections at 90 degrees of arcs of circles having various radii. The angle between the diverging curves appears greater than 90 degrees, while the angle between the converging curves appears much less than 90 degrees. If there is added to this the further apparent distortion of the angles by the curving axis of the upper femur, it is clear that confusion could easily arise as to the true angles between the systems of curved trabeculae. The correct method for the measurement of the angle between two curved lines is to draw through their intersection straight lines perpendicular to the radius of each curve; the angle between these two intersecting straight lines is the angle between the two curves at their intersection.

b. The principal compressive group. This group of trabeculae (figs. 21 and 28) springs from the medial portion of the shaft just above the group above-described, and spreads upward and in slightly radial smooth curved lines to reach the upper portion of the articular surface of the head of the femur. These trabeculae are placed very closely together and are the thickest ones seen in the upper femur. They are a prolongation of the shaft, from which they spring in straight lines which gradually curve to meet at right angles the articular surface.

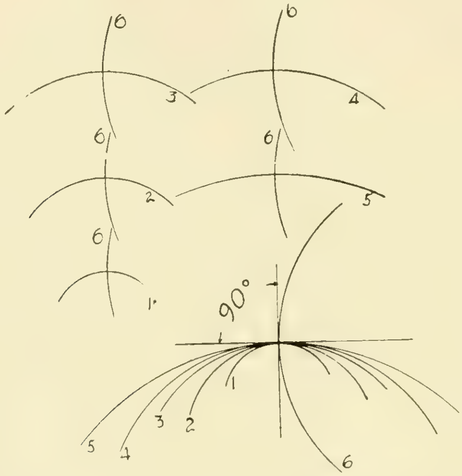


Fig. 22 Diagram of curves of different radii, intersecting at right angles. Detailed discussion of this figure is given in Part IV, under 'The Secondary Compressive Group.' (P. 253.)

There is no change as they cross the epiphyseal line. They also intersect at right angles the system of lines which rise from the lateral side of the femur. There are a few light trabeculae of this group which rise along the lower portion of the neck of the femur and end below the fossa of the ligamentum teres.

This system of principal and secondary compressive trabeculae corresponds in position and in curvature with the lines of maximum compressive stress, which were traced out in the mathematical analysis of this portion of the femur (fig. 19).

B. Lateral (tensile) system of trabeculae. As the compact bone of the outer portion of the shaft approaches the greater trochanter it gradually decreases in thickness. Beginning at a point about 1 inch below the level of the lower border of the greater trochanter, numerous thin trabeculae are given off from the outer portion of the shaft. These trabeculae lie in three distinct groups: one (c) which rises from the upper part of the shaft and passes almost vertically and ends in the surface of the greater trochanter: the second (d) which rises lower in the shaft than the group just mentioned, making an angle of 30 to 45 degrees with the shaft, and crosses in almost parallel curved lines the neck of the femur and ends in the medial surface of the head of the femur: the third (e), which rises from the same side of the shaft and below group (d) and crosses the longitudinal axis of the femur and ends in the medial side of the neck and shaft.

c. The greater trochanter group. These trabeculae rise from the outer part of the shaft just below the greater trochanter and rise in thin, curving lines to cross the region of the greater trochanter and end in its upper surface. Some of these filaments are poorly defined. This group intersects the trabeculae of group a which rise from the opposite side. The trabeculae of this group evidently carry small stresses as is shown by their slenderness.

d. The principal tensile group. This group springs from the outer part of the shaft immediately below group c, and curves convexly upward and inward in nearly parallel lines across the neck of the femur and ends in the inferior portion of the head. These trabeculae are somewhat thinner and more widely spaced than those of the principal compressive group (b). All the trabeculae of this group cross those of groups a and b at right angles. This group is the most important of the lateral system (tensile) and, as will be shown later, the greatest tensile stresses of the upper femur are carried by the trabeculae of this group.

e. The secondary tensile group. This group consists of the trabeculae which spring from the outer side of the shaft and lie below those of the preceding group. They curve upward and medially across the axis of the femur and end more or less irregularly after crossing the mid line, but a number of these filaments end in the medial portion of the shaft and neck. They cross at right angles the trabeculae of group a.

C. General. The description of the inner architecture of the middle frontal section given above applies to the parallel serial sections, which differ from it because the latter sections include increasing amounts of the compact bone of the outer shell, as the limits of the bone anteriorly and posteriorly are approached.

2. The inner architecture of the sagittal mid-section. Figure 23 represents a sagittal section through the upper femur. This specimen was made from a femur from the dissecting room, and is typical of the structure of numerous other normal femurs that have been examined during the preparation of this paper. This section has been cut so as to include the neutral plane of the upper femur and therefore gives a good representation of the architecture of the femur in the region of the neutral axis.

The portion of the section that passes through the head consists of a fine mesh-work of spongy bone of very uniform density. This mesh-work is produced by the intersection of two groups of trabeculae which rise from the compact bone of the anterior and posterior portions of the neck and head of the femur, and cross the central part of the head to end on the side opposite. The articular surface of the head of the femur is a thin shell of compact bone.

The trabeculae in the region immediately below the head rise from the anterior and posterior portions of the shaft in curving, wide-spaced, almost vertical lines, with an irregular cross-bracing of thin trabeculae. As the posterior portion of the shaft of the femur approaches the lesser trochanter from below, it gradually becomes thinner, and the trabeculae rise from it in straight, parallel lines which cross the region of the lesser trochanter, and above it fuse together to form an outer shell of compact bone in the neck. This shell is reinforced by a similar fusing of some of the trabeculae above the lesser trochanter to form a single spur-like line of compact bone parallel to the longitudinal axis of the femur. This spur becomes thicker as it approaches the neck.

The trabeculae lying between the anterior part of the shaft and the prolongation of the posterior portion of the shaft are thin and wide-spaced. Those lying in the space occupied by the lesser trochanter are thin and more closely spaced, and are parallel to the longitudinal axis of the femur. The latter are

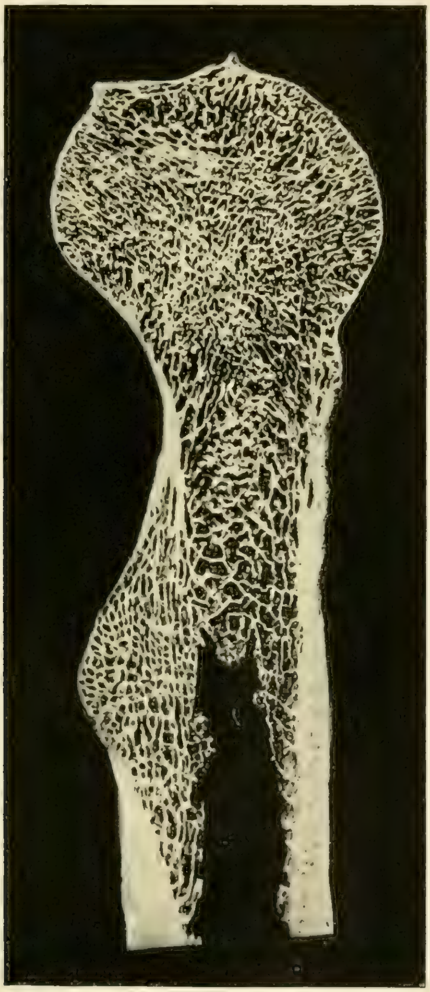


Fig. 23 Sagittal mid-section of upper femur. This is taken from a dissecting-room specimen of normal structure. The sagittal mid-section of the lower end of this femur is shown in figure 26. (Natural size.)

crossed at right angles by a more widely spaced system of trabeculae.

A. Structural features. The structural features of most importance in this section are:

1. The spongy bone is most closely arranged in the head of the femur, and it becomes thinner and more widely spaced as the lesser trochanter is approached.

2. The thinning of the trabecular structure begins immediately below the articular surface of the head.

3. The change of the heavy compact bone, on the posterior side below the trochanter, to the system of light trabeculae which crosses the lesser trochanter and fuses to form the compact bone of the neck of the femur.

3. The serial transverse sections of the upper femur. T ese sections made at $\frac{1}{4}$-inch intervals, are so numbered that the distance in quarter-inches, measured along the neutral axis, from the proximal end is indicated by the number of the section.

In Plates 1 and 2 only the alternate transverse sections of the upper femur are shown. These sections from 2 to 20 confirm the observation made of the sagittal section, previously described: that the trabecular structure distal to the head gradually becomes lighter after the lower level of the articular surface of the head is passed.

The cross section of the head of the femur is practically circular, and it is made up entirely of spongy bone except for the thin shell of compact bone forming the articular surface. Sections through the neck show a gradual thickening of the outer shell to form the shaft, with a parallel decrease in the density of the spongy bone, as the sections are taken more and more distal to the head. In the neck there is a marked decrease in the gross area of the cross section, which reaches a minimum in section 8. Referring to table 5, it will be seen that the area of compact bone in this section, including the equivalent area of the spongy bone, is less than that of any other section of the entire femur. The marked variations not only of relative density of the spongy bone, but of the size and shape of cross section that are seen in the successive sections of the head and neck are of great importance in determining the mechanical strength of this part of the femur. Transverse sections afford the most accurate means of computing the strength of the bone at various sections. In Part III the mechanical analysis of these separate sections has been made, and the results are presented in table 5. Figure 20 gives in diagrammatic form the strength of the femur about its two principal axes *A–A* and *B–B*.

The shaft of the femur

The shaft will be considered here as that portion of the femur lying between the lower limit of the lesser trochanter and the distal 6 inches of the femur.

1. Frontal sections. The shaft consists of a thick, hollow, cylindrical shell of compact bone with relatively unimportant short, thick trabeculae projecting from the inner surface of the shaft. The central core of the shaft is occupied by very fragile trabeculae which support the marrow in this cavity, but which probably do not normally affect the strength of the femur.

The frontal longitudinal sections of the shaft, being cut through a more or less circular hollow cylinder, do not give an adequate picture of the structure because large variations occur in the form of the sections as they are taken at a distance from the center. Figure 24 gives a general idea of the structure of the shaft, but the most accurate representation of the shaft is given by the serial transverse sections, shown in Plates 1–3.

Figure 24 clearly shows the gradual fusion of the trabeculae in the head and neck to form the shaft, and at the lower end the gradual thinning of the shaft which takes place with a corresponding increase in the amount of spongy bone.

2. Sagittal sections. Longitudinal sagittal sections of the shaft proper are somewhat similar in general appearance to the frontal sections, as they are cut in the longitudinal plane at right angles to the frontal sections. But they give only a general idea of the structure of the shaft, as they become distorted as they are taken in planes away from the center.

3. Serial transverse sections. These sections through the shaft are shown in Plates 1–3 (sections 20–52). They give the detailed

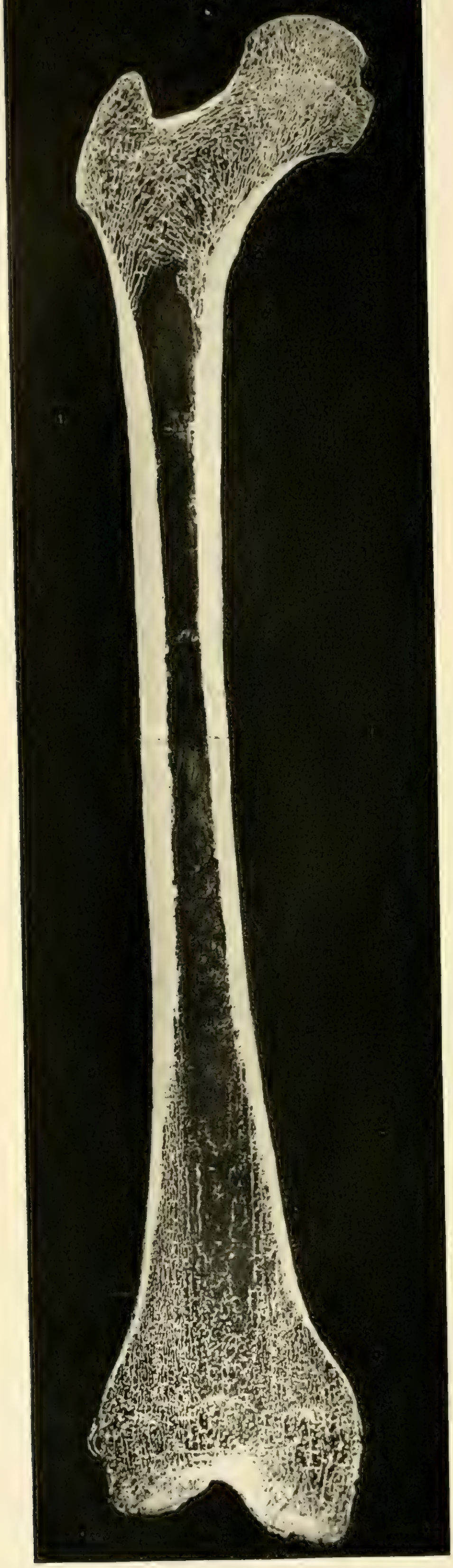


Fig. 24 Frontal longitudinal mid-section of left femur. Taken from same subject as figures 21 and 25 and Plates 1–5. (4/9 of natural size.)

architecture of the shaft without distortion and offer the most accurate basis for determining the true strength of this part of the femur.

The shape of section 20 is almost circular, with a similar hollow space within. Distal to this section the shape of the shaft gradually changes, becoming somewhat elliptical in section 28. The spongy bone in the sections between 20 and 28 becomes markedly smaller in amount, in section 28 only part of the area within the compact shell being occupied by spongy bone. Beyond section 28 the area of spongy bone is so small as to be negligible in computing the strength of the bone, until the distal part of the femur is reached.

The outline of the shaft from section 32 to section 52 is pear-shaped, with a fairly symmetrical hollow space within, which produces an annular figure of variable thickness.

The chief value of the transverse sections is that they give an accurate basis for computing the strength of the shaft, and they give the true outlines of the femur at successive sections.

The distal portion of the femur

This portion includes all of the femur distal to section 52 where the shaft begins to increase in gross area of cross section.

1. Frontal section. Figure 25 shows the middle frontal section through the femur. There are seen to be two main systems of trabeculae, a longitudinal and a transverse system. The trabeculae of the former rise from the inner wall of the shaft and continue in perfectly straight lines parallel to the axis of the shaft and proceed to the epiphyseal line, whence they continue in more or less curved lines to meet the articular surface of the knee-joint at right angles at every point. Near the center there are a few thin, delicate, longitudinal trabeculae which spring from the longitudinal trabeculae just described, to which they are joined by fine transverse filaments that lie in planes parallel to the sagittal plane.

The trabeculae of the transverse system are somewhat lighter in structure than those of the longitudinal system, and consist of numerous trabeculae at right angles to the latter.

As the distal extremity of the femur is approached the shaft gradually becomes thinner until the articular surface is reached, where there remains only a thin shell of compact bone. With the gradual thinning of the compact bone of the shaft, there is a simultaneous increase in the amount of the spongy bone, and a gradual flaring of the femur which gives this portion of the bone a gradually increasing gross area of cross section.

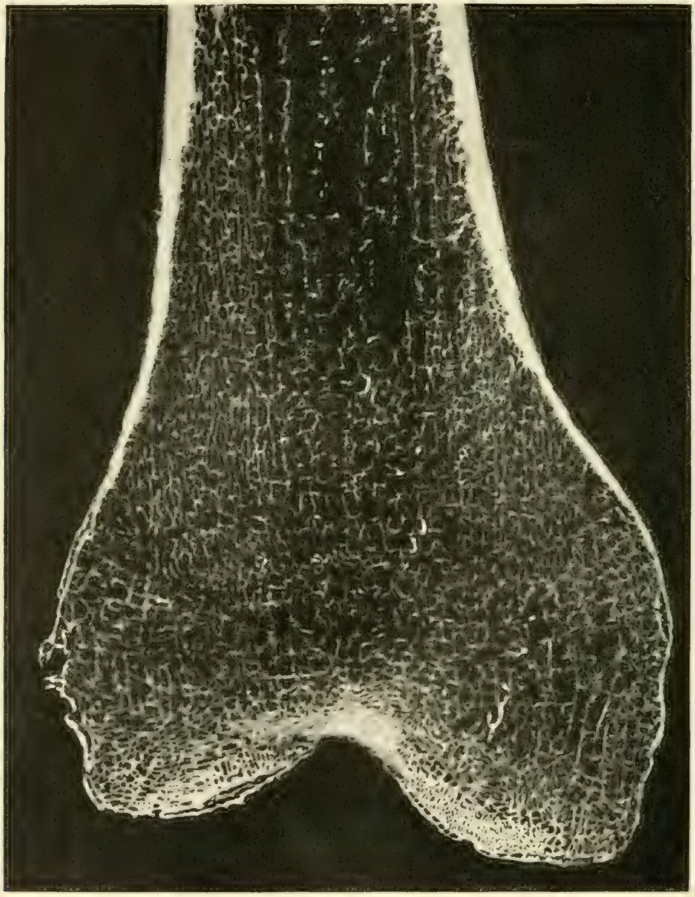


Fig. 25 Frontal longitudinal mid-section of distal part of left femur. Taken from same subject as figures 21 and 24. (Natural size.)

There is a marked thickening of the shell of bone in the region of the intercondyloid fossa where the anterior and posterior crucial ligaments are attached. This thickened area is about 0.4 inch in diameter and consists of compact bone from which a number of thick trabeculae pass at right angles to the main longitudinal system. The inner structure of the bone is here evidently adapted to the efficient distribution of the stresses arising from these ligamentary attachments.

Near the distal end of the femur the longitudinal trabeculae gradually assume curved paths and end perpendicularly to the articular surface at every point.

Such a structure is in accordance with the principles of mechanics, as stresses can be communicated through a frictionless joint only in a direction perpendicular to the joint surface at every point.

2. Sagittal sections. The sagittal mid-section of the distal portion of the femur is shown in figure 26. It shows two systems of trabeculae, which spring from the anterior and posterior portions of the compact bone of the shaft, which becomes thinner as the joint-surface is approached. Fine trabeculae are given off from the posterior part of the shaft and after crossing the central area in curving paths and perpendicularly in the anterior articular surface.

The second system of trabeculae rises from the anterior part of the shaft about 3 inches from the joint, and ends partly on the anterior, but chiefly on the posterior surface of the joint. These trabeculae all end in lines perpendicular to the joint-surface.

3. Serial cross sections. The serial cross sections from 52 to 75, Plates 3 to 5 show the gradual increase in the gross cross sectional area of the femur as the knee-joint is approached. Taken with the longitudinal frontal and sagittal sections already described, these sections give a very clear idea of the internal architecture. Beginning with section 52 the amount of spongy bone gradually increases, but not until section 58 is reached is the entire inner area completely occupied by trabeculae. This corresponds closely with the longitudinal frontal section. As the area occupied by the spongy bone increases the thickness of the shell of compact bone decreases, finally reaching a minimum in section 75.

In sections 68 to 75 two systems of trabeculae are seen: one consisting of lines paralleling on either side of the center, the lateral and medial margins of the sections; the other, a sys-

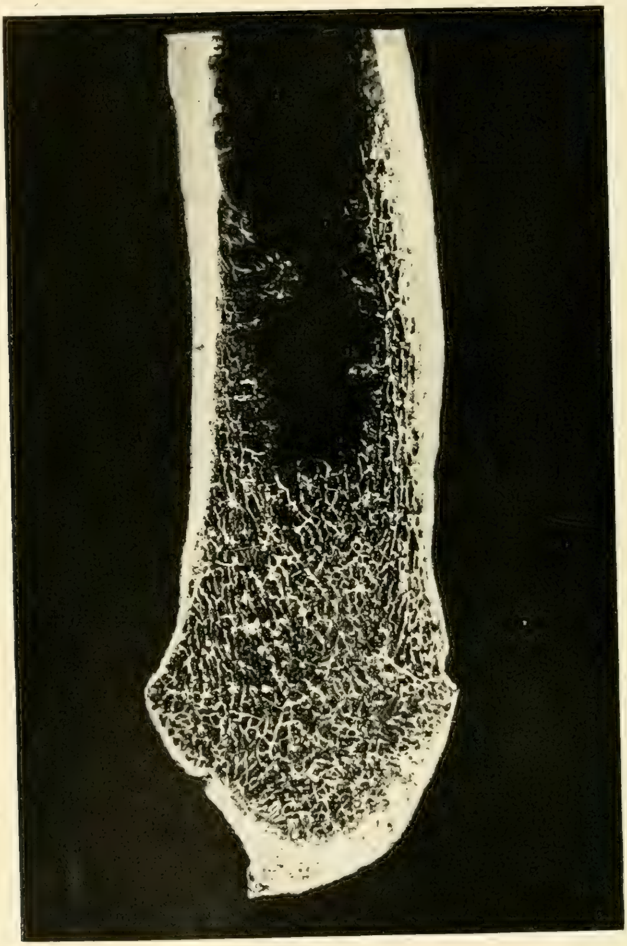


Fig. 26 Sagittal mid-section of distal end of femur. This is taken from the dissecting room specimen of normal structure, the upper end of which is shown in figure 23. (Natural size.)

tem which is transverse to the first system, and which is arranged largely in the form of radial lines.

4. Structural features. The essential features of the distal part of the femur are the following:

1. Gradual increase in the total area of the cross section with practically no increase in the total equivalent area as compact bone, from sections 52 to 70 inclusive, as shown in table 5, column 7 (p. 235).

2. The system of longitudinal trabeculae parallel to the longitudinal axis of the femur. These trabeculae are braced by two other systems of transverse trabeculae, which are practically at right angles to each other and to the longitudinal system.

3. With practically no increase in the amount of bony material used, there is a greatly increased stability produced by the expansion of the lower femur from a hollow shaft of compact bone to a structure of much larger cross section almost entirely composed of spongy bone.

PART V. SIGNIFICANCE OF THE INNER ARCHITECTURE OF THE FEMUR

General

In Part III the femur was analyzed in detail by the methods of applied mechanics for an assumed load of 100 pounds on the femur-head. Within the elastic limit of materials the stresses produced by any given load are proportional to the load: a load of 100 pounds will produce stresses one hundred times as great as those produced by a load of one pound. Hence the analysis based on the assumed load can be readily adapted by simple proportion to yield correct values of the stresses produced by other loads, provided the elastic limit is not exceeded.

For these reasons the lines of maximum stress within the femur are in the same position for a load of one pound or of 100 pounds, or any other load that does not stress the bone beyond the elastic limit. As the elastic limit for almost all materials is about one-half of the ultimate or breaking strength of the material, these conclusions will apply to all loads ordinarily carried by the femur. The discussion of the factors of safety of the femur will be taken up in later paragraphs.

The detailed description of the inner architecture has been given. The comparison of the mathematical analysis of the femur with the inner architecture of the bone will be discussed, the upper femur, shaft and distal portion being considered separately.

Significance of the inner architecture of the upper femur

1. Frontal sections. Figure 21 shows a longitudinal frontal section through the upper left femur, which is the mate of the right femur on which the mathematical analysis was made. In this mid-section the system of tensile trabeculae, which rises from the lateral (outer) part of the shaft and crosses over the central area to end in the medial portion of the shaft, neck and head, is clearly shown. This figure also shows the compressive system of trabeculae which rises on the medial portion of the shaft and crosses the central area to end in the head, neck and greater trochanter. By comparing the position of these two systems of trabeculae shown in figure 21 with the lines of maximum and minimum stresses shown in figure 19, it is seen that the tensile system of trabeculae corresponds exactly with the position of the lines of maximum and minimum tensile stresses which were determined by mathematical analysis. In a similar manner, the compressive system of trabeculae in figure 21 corresponds exactly with the position of the lines of maximum and minimum compressive stresses computed by mathematical analysis. It may be explained, that the maximum compressive stresses are medial to the longitudinal axis; the minimum compressive stresses are lateral to the longitudinal axis. Also, the maximum tensile stresses are lateral to the longitudinal axis, and the minimum tensile stresses are medial to this axis. These relations are shown graphically in figure 19 *a*, in which the approximate intensity of the tensile and compressive stresses at various points is shown together with the direction in which these stresses act at the given points.

It has been shown that, in general, the trabeculae of the tensile system are lighter in structure than those of the compression system in corresponding positions. The significance of the difference in thickness of these two systems is that the thickness of the trabeculae varies with the intensity of the stresses at any given point. Comparison of figure 21 (also see fig. 28) with figure 19 *a*, will show that the trabeculae of the compression system carry heavier stresses than those of the tensile system in corresponding positions. For example, the maximum tensile stress at section 8 (fig. 19 *a*) in the outermost fiber is 771 pounds per square inch, and at the corresponding point on the compression side the compressive stress is 954 pounds per square inch. Similar comparisons may be made at other points, which will confirm the conclusion that the thickness and closeness of spacing of the trabeculae varies in proportion to the intensity of the stresses carried by them.

As the trabeculae of both systems approach the level of the lesser trochanter, they become thinner and farther apart. The explanation for this lighter structure is seen in the much lower stresses in both systems in this region, as shown in figure 19 *a*.

It will be seen that the trabeculae lie exactly in the paths of the maximum tensile and compression stresses (compare figs. 21, 19 and 19 *a*), and hence these trabeculae carry these stresses in the most economical manner. This is in accordance with the well-recognized principle of mechanics that the most direct manner of transmitting stress is in the direction in which the stress acts.

Further significance of the spongy structure of the upper femur

The question may well be asked, Why should the head and neck of the femur be composed so largely of spongy bone, with a gradual transition to compact bone in the hollow shaft? Referring to figure 16, it is seen that the amount of the vertical shear varies almost uniformly from a maximum of 90 pounds (90 per cent of the load on the femur-head) midway between sections 4 and 6, to a minimum of – 5.7 pounds at section 18.

The effect of vertical shearing force, as explained in Part II, (*50*), in such a structure as the femur, is to produce maximum horizontal shearing stresses and maximum vertical shearing stresses on the neutral plane. The magnitude of these maximum shearing stresses on the neutral plane is about 1.4 times as great as the average vertical shearing stress on the entire cross section. Referring to table 5, column 6, it is seen that the equivalent area of the spongy bone, converted into terms of compact bone in the upper femur, varies from a maximum of 0.816 square inch at section 4, to a minimum of 0.022 square inch at section 18. This gradual and consistent diminution of the spongy bone parallels the diminishing intensity of the vertical shear. Hence it may be concluded that one of the functions of the spongy bone in the upper femur is to resist the shearing forces and that the amount of the spongy bone in this region varies with the amount of the shearing stresses.

Conclusions. It may therefore be concluded from the foregoing that:

1. The trabeculae of the upper femur, as shown in frontal sections are arranged in two general systems, compressive and tensile, which correspond in position with the lines of maximum and minimum stresses in the femur determined by the mathematical analysis of the femur as a mechanical structure.
2. The thickness and spacing of the trabeculae vary with the intensity of the maximum stresses at various points in the upper femur, being thickest and most closely spaced in the regions where the greatest stresses occur.
3. The amount of bony material in the spongy bone of the upper femur varies in proportion to the intensity of the shearing force at the various sections.
4. The arrangement of the trabeculae in the positions of maximum stresses is such that the greatest strength is secured with a minimum of material.

Significance of the inner architecture of the shaft

1. Economy for resisting shear. The detailed description of the inner architecture of the femur has been given in a preceding section. By referring to figure 16, it will be seen from the "Curve of vertical shear" that the shearing forces are a minimum between sections 16 and 18, and for all points distal to the latter section the shearing force is the same as at section 18. Hence, for all points in the femur below this section the shearing stresses will also be very near a minimum. It is clear that a minimum amount of material will be required to resist the shearing stresses distal to section 18. As horizontal and vertical shearing stresses are most efficiently resisted by material placed near the neutral plane, in this region a minimum amount of material will be needed near the neutral axis. Referring to Plates 2 and 3, sections 20–52, it will be seen that the cross sections have little if any material in the central space in the shaft, practically the only material near the neutral plane being in the compact bone, but lying at a distance from the neutral axis. This conforms to the requirement of mechanics for economy, as a minimum of material is provided for resisting shearing stresses where these stresses are a minimum. The load of 100 pounds on the femur-head produces horizontal and vertical shearing stresses on the neutral plane of − 11 pounds per square inch in all the sections distal to section 18 (table 6, cols. 12, 13). These stresses are amply provided for by the compact bone lying in the neutral plane.

2. Economy for resisting bending moment. In figure 16, the "Curve of bending moments" shows that the amount of the bending moment increases from a minimum at section 4 to a maximum between sections 16 and 18, then gradually decreases almost uniformly to 0 near section 75. The measure of the resistance of a section to bending moment is the section modulus. To resist bending moment stresses most effectively the material should be as far from the neutral axis as possible. For equal areas the section moduli vary directly as the distance at which the entire area may be considered as concentrated. Hence the farther the material lies from the central axis the more effective its resistance to bending moment stresses. It is evident that the hollow shaft of the femur is an efficient structure for resisting bending moment stresses, all of the material in the shaft being relatively at a considerable distance from the neutral axis. It is evident that the hollow shaft provides efficiently for resisting bending moment not only due to the load on the femur-head, but from any other loads tending to produce bending in other planes.

3. Economy for resisting axial stress. The relatively small axial stress, which is compressive at all points in the femur and is uniformly distributed over the area of each cross section, is efficiently provided for by the compact bone of the shaft, as may be seen by reference to figure 18 and 18 *a* and table 6.

4. Conclusions. From the foregoing it may be concluded that:

1. The inner architecture of the shaft is adapted to resist in the most efficient manner the combined action of the minimal shearing forces and the axial and maximum bending stresses.

2. The structure of the shaft is such as to secure great strength with a relatively small amount of material.

Significance of the inner architecture of the distal part of the femur

1. Relation between structure and function. The function of the lower end of the femur is to transmit through a hinged joint the loads carried by the femur. For stability the width of bearing on which the hinge action occurs should be relatively large. For economy of material the expansion of the end bearing should be as lightly constructed as is consistent with proper strength. In accordance with the principles of mechanics already discussed, the most efficient manner in which stresses are transmitted is by the arrangement of the resisting material in lines parallel to the direction in which the stresses occur and in the paths taken by the stresses. Theoretically the most efficient manner to attain these objects would be to prolong the innermost filaments of bone as straight lines parallel to the longitudinal axis of the bone, and gradually to flare the outer shell of compact bone outward, and continuing to give off filaments of bone parallel to the longitudinal axis as the distal end of the femur is approached. These filaments should be well-braced transversely and each should carry its proportionate part of the total load, parallel to the longitudinal axis, transmitting it eventually to the articular surface, in a direction perpendicular to that surface.

Referring to figure 25, which shows the essential features of the lower femur in longitudinal frontal section, it is seen that the large expansion of the bone is produced by the gradual transition of the hollow shaft of compact bone to cancellated bone, resulting in the production of a much larger volume. The trabeculae are given off from the shaft in lines parallel to the longitudinal axis, and are braced transversely by two series of trabeculae at right angles to each other, in the same manner as required theoretically for economy. This construction is an excellent illustration of the principle that a long, slender column (as a single trabecula) braced at frequent intervals, acts as a short column whose height is the distance between the braces. The strength of the femur where the spongy bone forms a large part of the cross section, is somewhat reduced, because the trabeculae are not capable of carrying quite as large stresses as an equal amount of bone in a compact mass. The material in spongy bone comprising the system of transverse bracing is not effective in carrying the stresses in a longitudinal direction, while in the compact bone practically all of the material is effective in transmitting stress in a longitudinal direction.

Referring to figure 17 (also see fig. 18), where the maximum unit-stresses in the femur are given in diagrammatic form, for a load of 100 pounds on the femur-head, it will be seen that the maximum unit-stresses at section 52 are 817 pounds compression and 465 pounds per square inch tension, on the medial and lateral sides, respectively. It will be seen that the intensity of these maximum unit-stresses decreases rapidly to 356 pounds compression and 8 pounds per square inch tension, respectively at section 64. Distal to this section (64) the stresses on both medial and lateral sides of the neutral axis becomes compressive and gradually approach the value of 65 pounds per square inch uniformly distributed over section 75.

The actual compressive strength of the lower femur decreases gradually as the compact bone is replaced by spongy bone. This decrease in strength roughly parallels the decrease in the intensity of the maximum unit-stresses as the distal end is approached. The considerable increase in the gross area of the transverse sections of this portion of the femur is secured with practically no increase in the amount of bony material in these sections until section 72 is reached. While the direct compressive strength of the lower femur is somewhat decreased by the change to spongy bone, the stiffness or resistance to bending in any direction is increased greatly. The stiffness of the femur at section 75 is more than five times as great as that of section 52, although the area of the cross section in terms of compact bone is only slightly more than double that of the latter. In the distal portion of the femur in which the expansion takes place, there is no increase in the amount of bony material used until the lowermost 1.25 inches is reached.

A well-recognized principle in mechanics is illustrated by the expanded lower end of the femur, by means of which the load transmitted from the femur to the tibia is evenly distributed over an area much greater than that of the shaft through which the load has passed, thereby reducing the danger of rupture at the joint. This is analogous to the foundation of large area upon which all columns rest, in order to distribute the concentrated load over an area having a comparatively low supporting power. By means of this expanded bearing there is greater stability at the joint, and greater resistance against lateral bending.

2. Conclusions. 1. The inner architecture of the distal portion of the femur is well adapted for the diminishing intensity of the direct stresses, to which it bears a somewhat parallel relation.

2. The loads transmitted through the femur are distributed in a manner that conforms to the requirements of mechanics in securing strength and stability with economy of material.

3. The inner structure of this portion of the femur is economically adapted to the mechanical requirements of this portion of the femur.

Summary—laws of bone structure

In the preceding paragraphs of this section it has been shown that in every part of the femur there is a remarkable adaptation of the inner structure of the bone to the mechanical requirements due to the load on the femur-head. The various parts of the femur taken together form a single mechanical structure wonderfully well-adapted for the efficient, economical transmission of loads from the acetabulum to the tibia; a structure in which every element contributes its modicum of strength in the manner required by theoretical mechanics for maximum efficiency.

It has been indicated that, in each of the arbitrary divisions into which the femur was divided for clearness of discussion, the internal structure is everywhere so formed as to provide in an efficient manner for all the internal stresses which occur due to the load on the femur-head. Throughout the femur, with the load on the femur-head, the bony material is arranged in the paths of the maximum internal stresses, which are thereby resisted with the greatest efficiency, and hence with the maximum economy of material. The conclusion is inevitable that the inner structure and outer form of the femur are governed by the conditions of maximum stress to which the bone is subjected normally by the preponderant load on the femur-head; that is, by the body weight transmitted to the femur-head through the acetabulum. In the normal individual the maximum load on the femur-head occurs in running.

In this paper it has been shown that the femur obeys the mechanical laws that govern other elastic bodies under stress; the relation between the computed internal stresses due to the load on the femur-head, and the internal structure of the different portions of the femur is in very close agreement with the theoretical relations that should exist between stress and structure for maximum economy and efficiency: and therefore, it is believed that the following laws of bone structure have been demonstrated for the femur:

1. The inner structure and external form of human bone are closely adapted to the mechanical conditions existing at every point in the bone.

2. The inner architecture of normal bone is determined by the definite and exact requirements of mathematical and mechanical laws to produce a maximum of strength with a minimum of material.

Further, the observations here recorded for the femur, the largest and heaviest bone of the body, must in a general way hold true for all the bones of the body: else we must assume the absurd conclusion that the structure of the femur is in conformity with mechanical laws and the other bones are based upon other unknown laws. The numerous experiments by Rauber and Messerer in testing human bone to destruction have shown that the physical properties of all the larger bones of the body are substantially the same. Hence, the laws formulated above for the femur must hold in general for all the bones of the body.

PART VI. MISCELLANEOUS AND CONCLUSIONS

The factor of safety in the human femur

Factor of safety, working stress. To assure the safety of any structure there must be no danger of breaking in any part under the heaviest loads for which the structure is designed. The greatest stress induced in any part of a structure by the heaviest loads which it will be called upon to carry must never approach the breaking strength of the material. The working stress for any material is that unit-stress (pounds per square inch, kilograms per square centimeter) which has been found safe to permit in that material and provide a necessary degree of security against breaking. Usually working stresses are determined by experiment. The working stress is the unit-stress employed in determining the sizes of structural members of any given material. The factor of safety is the ratio of the ultimate strength of the material to the working stress.

In the design of structures the purpose of the factor of safety is to guard against the unavoidable defects in the structural material employed which might reduce the ultimate strength of the material, and also to provide for the possibility of an unforeseen increase in the load to be carried. In wooden structures the factor of safety is usually about 10, in steel from 4 to 5.

It has been shown in Part III that in the erect, standing attitude the load resting on each femur could be taken at three-tenths the body weight: and in walking the loaded femur carried approximately eight-tenths of the body weight. In walking the load is carried alternately by the femurs, and it may be assumed that the load is cushioned by the padding of the feet and by muscle action so that it acts practically as a load gradually applied and produces the same effect as a static load of the same amount.

In running, jumping and falling, it is clear that the effect of such suddenly applied loads on the femur-head is greater than that due to standing or walking. It is a general principle of mechanics that the stresses produced by a suddenly applied load, without impact, are just double those produced by a gradually applied, or a static load of the same amount. Hence, if allowance is made for the cushioning effect of the padding of the feet and the resilience of muscles, ligaments, etc., as previously shown in Part III, the stresses produced in running may safely be taken as twice as great as those produced by walking. The femur is therefore considered to be stressed in running, as though it carried double the static load in walking, or a load of 1.6 times the body weight.

In jumping the stresses are still further increased by the impact of the body, which is suddenly brought to a stop. The velocity which the body attains just before striking determines the amount of the impact. The stresses produced by impact, as in jumping or falling, may be enormously greater than in walking or running. Probably few fractures occur without the element of impact being involved to a marked degree. As the mechanical analysis of the stresses due to impact holds only for stresses within the elastic limit, the large stresses due to impact are difficult to analyze. Impact may produce dynamic stresses many times as great as those produced by the static load alone. In the body the effect of impact is often offset to a greater or less degree by the involuntary flexing of the body at hips, knees and ankles as well as by the muscle tonus. For these reasons, probably, relatively few fractures result from the impact due to high jumps and falls.

In the femurs studied, the body weight being 200 pounds, the load carried by the loaded femur under various conditions discussed is as follows:

CONDITION	LOAD, IN TERMS OF BODY WEIGHT	WEIGHT
		pounds
Standing	0.30	60
Walking	0.80	160
Running	1.60	320
Jumping	Variable, but greater than for running	
Falls	Variable, but greater than for running	

This furnishes a basis for an approximation of the factor of safety in the femur. By referring to figure 17 it is seen that the greatest unit-stresses in both tension and compression occur at section 8, which is therefore the weakest section of the entire femur, when carrying a load on the femur-head in the normal manner. The maximum stresses at section 8 for a load of 100 pounds on the femur-head are 1310 and 974 pounds per square inch, in compression and tension, respectively. The maximum stresses, under normal conditions, are those due to running and are equal to those produced by a static load of 320 pounds on the femur-head. The maximum stresses at section 8 due to the load of 320 pounds will be 3.2 times as great as for the 100-pound load; or 4192 and 3117 pounds per square inch in compression and tension, respectively The excellent physical condition and comparative youth of the subject from whom the femurs analyzed were taken, warrants using the higher values of the ultimate strength of compact bone, as determined by actual tests by Rauber and other investigators. For this reason in the determination of the factor of safety of the femurs analyzed the values 24,000 and 17,700 pounds per square inch in compression and tension, respectively, will be used. The factor of safety at section 8 will be the smaller of the values found by dividing the ultimate strengths of compact bone by the maximum stresses at this section. The factor of safety in compression is thus found to be 5.71, and for tension it is 5.68; therefore the factor of safety for the stresses due to running is 5.68, which is also the factor of safety of the entire femur as it is the factor of safety of the weakest section of the femur.

The middle third of the femur is more exposed to injury from blows, falls and other accidents than other parts of the femur. For this reason the factors of safety of the bone in this region will be of considerable interest. Referring to figure 17, it will be seen that in sections 20 to 44, comprising the middle third, the unit-stresses are relatively high and at sections 20, 28 and 36 approach the maximum values reached at section 8. Based on the ultimate strength of compact bone at 24,000 and 17,700 pounds per square inch for compression and tension, respectively, the following factors of safety are found for the various sections of the middle third for the stresses due to running:

The minimum factor of safety is 5.98 at section 28; the maximum factor of safety in this region is 7.68 at section 44: the average value of the factor of safety is 6.60. Thus the factor of safety in the middle third is from 6 to 35 per cent greater than at the weakest section in the neck of the femur. These factors of safety are all based on the stresses due to running, with the load on the femur-head as in life.

Blows striking the femur will be at an angle to its axis, the maximum destructive effect being produced when the blow is directed at right angles to the axis of the bone. In the standing, walking and running positions nearly all blows will be received from a direction other than medial. Hence, such blows will tend to produce bending in the femur in a direction opposite to that produced by the load carred on the femur-head, so that these bending stresses of opposite character tend to balance each other.

In figure 27 the factors of safety in successive sections of the femur are shown for the stresses due to running. Those due to walking and to the standing position are proportionally larger and therefore need not be shown.

In the figure the positions of the heavier concentrations of stress in the femur due to muscle action are shown as follows: at the greater trochanter, where the glutei, quadratus femoris

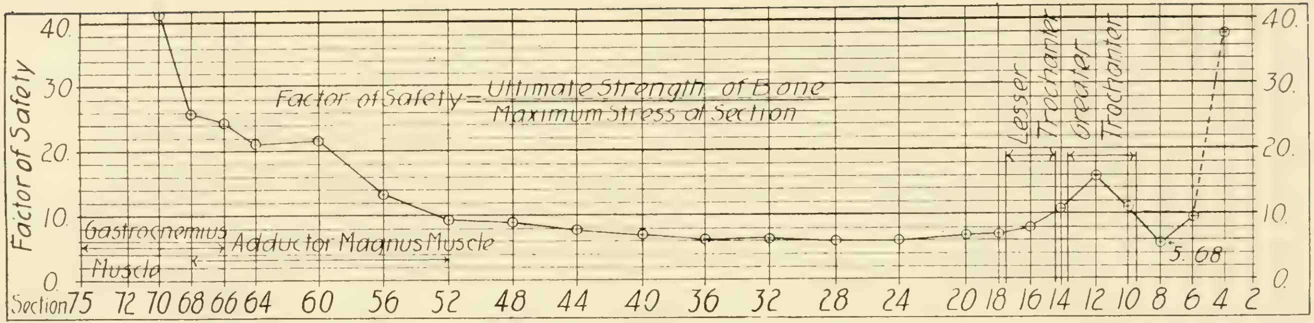


Fig. 27 Diagram of the factors of safety in the femur. In this diagram the ordinates represent the factor of safety, the abscissae represent the successive sections of the femur which have been analyzed in detail. The section numbers correspond with those shown in all the other diagrams. These factors of safety are for the stresses due to running, and are the lower of the values for the factor of safety in tension and compression, respectively, as tabulated in columns 20 and 21 of table 6. The locations of the insertions of the heavier muscles at the distal end and at the trochanters are shown, and in these regions the factor of safety is considerably increased above that of the adjacent regions.

and the group of smaller rotator muscles are inserted: at the lesser trochanter, where the ilio-psoas muscles are inserted: and at the distal part of the femur, where the gastrocnemius and adductor magnus muscles are inserted. It is very important to note that at these points of concentration of muscle action the factors

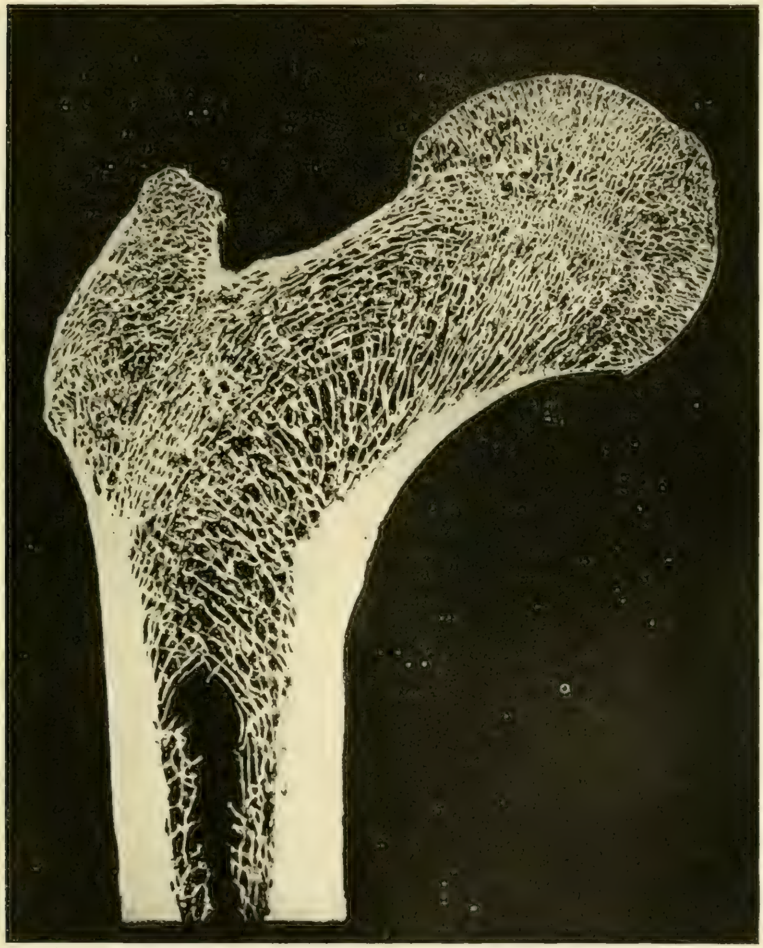


Fig. 28 Frontal longitudinal mid-section of upper femur. Dissecting room specimen. Taken from a femur picked up at random in the dissecting room. The analytic description given in the text for figure 21, applies to this section. (Natural size.)

of safety are in every case greater than in the adjacent sections. The increase in the factor of safety to about 10 in the region of the lesser trochanter is small as compared with the increase at the other points of concentration, and is to be accounted for by the fact that the muscle action here produces a tensile stress in the bone which is opposite in character to the compressive internal stress produced by the load on the femur-head. Hence, the two stresses tend to balance each other.

In the region of the greater trochanter the internal stress due to the muscle action is tensile as are also the internal stresses in this region due to the load on the femur-head: in this region it is seen that the factor of safety varies from 10.00 to 15.85, although in the neck where no muscles are attached the factor of safety is only 5.68.

At the distal end of the femur the factor of safety progressively increases as the extremity is approached. The increase follows approximately the magnitude of the muscles attached.

From a study of figure 27 it is evident that the larger muscles exert considerable influence on the structure of the bone as shown in the discussion above, and it is clear that additional strength is provided at the points where the larger muscular loadings occur. The actual factor of safety, therefore, is much more uniform for the normal load transmitted through the femur-head than is indicated by the curve plotted for the factor of safety in this figure.

It may be concluded that blows received with the femur carrying a load as in standing, walking or running, the maximum effect will be produced by a blow from the medial side and the minimum effect by a blow from the lateral side of the loaded femur.

It is evident from the larger factors of safety in the middle third that there is greater relative strength to resist blows at the middle third than in the upper femur.

The factor of safety of the femur for the greatest stress due to running has been shown to be 5.68; for walking it will be 11.36 and for the standing position it will be 30.30: these values are based on maximum values of the ultimate strength of bone.

Confirmatory data

1. Agreement of analysis with breaking tests. The analysis of the femur presented in the foregoing pages is in agreement quantitatively with the work done on the femur experimentally by Rauber ('76) and by Messerer ('80).

1. Breaking loads in tests on the femur made with testing machines by Messerer, with the load applied at the head of the femur and parallel to the axis of the bone were as follows:

25-year old woman	1350 kilos	=	2970 pounds
32-year old man	850 kilos	=	1870 pounds
78-year old man (heavy boned)	850 kilos	=	1870 pounds
82-year old woman	450 kilos	=	990 pounds

The calculated breaking load on the femur I analyzed in this paper would be 5.68 times 320 pounds or 1820 pounds. (The factor of safety being 5.68).

2. The loads at the middle required to break the femur in cross-bending tests made by Messerer are as follows:

24-year old man	750 kilos	=	1650 pounds
26-year old man	875 kilos	=	1930 pounds
31-year old man	1300 kilos	=	2850 pounds
24-year old woman	1100 kilos	=	2420 pounds
25-year old woman	875 kilos	=	1930 pounds

The calculated breaking load in cross bending for the femur I analyzed is as follows, based on an ultimate tensile strength of 17,700 pounds per square inch; and an ultimate compression strength of 24,000 pounds per square inch:

In frontal plane	2000 to 3200 pounds
In sagittal plane	2440 to 3310 pounds

The variations in the amount of the calculated load required to cause breaking is due to the variation in the resisting strength of the femur in the different planes about the axis of the femur.

There is very close agreement between these two series of tests and the calculated strengths based on my analysis. This agreement is as close as is to be expected in the case of other materials which are tested in a similar manner, such as steel, wood, etc.

3. Actual tests of the compact bone of the left femur analyzed in this paper (shown in figs. 21, 24 and 25) were made by me in the engineering testing laboratory of Johns Hopkins University. Pieces $1\frac{1}{2}$ inches long having a cross sectional area of 0.25–0.33 square inch broke at a maximum compression of 25,100 pounds per square inch.

4. If we compare the calculated maximum stresses as shown in figure 18 with the ultimate strength of compact bone in tension and in compression as determined by actual tests, a remarkable agreement is found in the ratios of the maximum tensile and compressive stresses. The ratio of the maximum tensile to the maximum compressive stress at successive sections of the femur (fig. 18) is everywhere less than 1.0 except at section 16 where the ratio is 1.010, at all other sections the ratio is between the limits of 0.926 at section 18 and 0.324 at section 60. The average of all the ratios of maximum tensile to maximum compressive stress is 0.652. The tests made by Rauber (1876) showed the ultimate strength of compact bone from 13,200 to 17,700 pounds per square inch for tension, and 18,000 to 24,000 pounds per square inch for compression. The ratios of these ultimate strengths are 0.733 to 0.738. The substantial agreement of the ratios of these theoretical tensile and compressive stresses with the ratio of the actual ultimate tensile and compressive strengths is of considerable value in checking the accuracy of the mathematical analysis quantitatively.

2. Distribution of fractures of the femur. Calculated probability of distribution of fractures of the femur. The theory of probability may be applied to the femur for the purpose of determining in what ratio fractures from blows should occur in various regions. Without entering into detail, such a study involves the assumption of the following rational premises:

1. The tendency for a fracture to occur at any point varies inversely as the least strength of the bone at that point.

2. Blows of the same intensity are equally apt to be received at any point along the femur. Although blows may vary in intensity, they are equally distributed.

3. The tendency to break at any point varies directly as the distance from the nearer support to the given point, the femur being assumed to be supported at both ends.

Upon these assumed conditions, calculations of the distribution of the breaks occurring from blows on the femur, reduced to percentages are as follows:

	PER CENT
Neck of femur	4.2
Upper third (exclusive of neck)	19.0
Middle third	60.2
Lower third	16.6
	100.0

A large number of breaks occurs in the neck from causes other than blows at an angle to the axis of the femur. For this reason consideration of the fractures in the neck will be omitted in this study. Then there results the following calculated distribution of fractures of the femur exclusive of those of the neck:

	PER CENT
Upper third	19.9
Middle third	62.9
Lower third	17.2
	100.0

The statistics of the location of fractures of the femur as given by Hyde ('75) for Bellevue Hospital are as follows:

		PER CENT
(Neck	61)	
Upper third (exclusive of neck)	34	14.0
Middle third	168	69.8
Lower third (including 7 condyles)	39	16.2
	241	100.0

Similar statistics given by Hamilton, quoted by Stimson ('12), are as follows:

		PER CENT
(Neck	84)	
Upper third	30	19.6
Middle third	86	56.7
Lower third	36	23.7
	152	100.0

If we combine these statistics of these two investigators we have the following:

		COMBINED PER CENT	COMPUTED PER CENT
Upper third	64	16.3	19.9
Middle third	254	64.6	62.9
Lower third	75	19.1	17.2
	393	100.0	100.0

The close agreement of the actual distribution of breaks of the femur with that determined by the application of the mechanical principles and the laws of probability, as I have indicated, is such as to be at least very suggestive as to the correctness of the analysis.

3. Summary. My analysis of the femur is found to be in agreement with the tests on longitudinal breaking and cross-breaking tests on femurs made carefully by Messerer: the computed maximum unit-stresses in the femur are closely parallel to the ultimate strengths of compact bone as determined by Rauber: the statistics of the location of fractures in the femur are very near the calculated distribution based on this analysis of the femur; and my own breaking tests on one of the femurs analyzed are in close agreement with Rauber's tests.

These independent checks on the soundness of this analysis of the femur, and the consistency of the quantitative relations established throughout, afford the strongest confirmation of the accuracy of the computations and the correctness of the applications of the principles of mechanics to this problem.

General comments

The doctrine of the functional form of bone, with its coralary of the functional pathogenesis of deformity, advanced by Wolff, and maintained by him with rare courage and persistence for so many years, is confirmed mathematically for the first time by the studies presented in this paper, for the structure of normal bone. To confirm the doctrine of the functional pathogenesis of deformity mathematically, there is needed only the application of the principles and methods used in this paper to the analysis of a suitable deformed bone, together with the clinical history of the case. The establishment of Wolff's doctrines upon a sound mathematical foundation should lead to a wider recognition of their importance and value both from the standpoint of the correction as well as of the prevention of deformity.

Homogeneity of spongy and compact bone

Gebhardt ('01) has advanced a number of ingenious arguments tending to prove that the spongy bone is not homogeneous with compact bone as a structural material and that they do not act together as a homogeneous structure. The close inter-relations between the spongy and the compact bone in the upper and lower femur, described in detail in Part IV, and the significance of their morphology described in Part V, proves that compact and spongy bone act together as a homogeneous structure. The analysis in Part III makes clear that unless the spongy bone possesses relatively the same strength as compact bone (weight for weight) these two types of structural material could not act together to form a homogeneous structure.

Summary

The general principles of applied mechanics and graphic statics are presented in brief form in Part II, and their practical application to the analysis of the structure of bone, and especially of the femur, is indicated and illustrated by specific examples.

The detailed mathematical analysis is made of the mechanics of the femur of a man in normal health who was accidentally killed and whose weight was 200 pounds. The structural properties of the femur sections are analyzed by graphic methods and the results shown in tables as well as by diagrams. The effect of an assumed load of 100 pounds acting on the femur-head in the same direction as the weight of the body, under normal conditions, is analyzed at intervals of $\frac{1}{2}$ inch in the extremities of the femur and at 1-inch intervals in the shaft. The amounts of the various stresses are computed for the assumed weight. The paths and amounts of the maximum internal stresses are computed in this normal femur and demonstrated to agree exactly with the position of the trabeculae in the upper femur.

The load on the femur-head in the normal, standing position is 0.3 of the body weight (on each femur): in walking, the weight carried alternately by the loaded femur-head is approximately 0.8 of the body weight: in running, the dynamic effect of the sudden application of the load produces stresses twice as great as in walking, or the effect is the same as double the static load of walking, or 1.6 times the body weight. In the particular case analyzed, the body weight being 200 pounds, the stresses in the loaded femur due to standing, walking and running are those due to a load on the femur-head of 60, 160 and 320 pounds, respectively.

Hence, to determine the stresses produced in standing, walking or running, the stresses produced in the femur at any section by the assumed load of 100 pounds are multiplied by 0.6, 1.6 and by 3.2, respectively.

The femur is shown to have the external form and internal structure to resist economically the stresses produced by the preponderant load, which is that on the femur-head due to the body weight in running. The spongy structure of the head of the femur resists in the most economical manner the vertical and horizontal shearing stresses which are greatest in the head and neck. In the shaft, where the shearing stresses are a minimum and the stresses due to bending moment relatively large, the compact bone of the hollow shaft resists these stresses most efficiently because the greater the distance of the bone from the neutral plane the greater the resisting strength of the bone. Finally, the large expansion of the lower end of the femur, to render the hinge-action of the knee joint strong against lateral bending, is produced by the transition of the compact bone to spongy bone requiring but a slightly greater amount of bony material for greatly increased stiffness. Thus the compact and spongy bone act in unison to produce the maximum of strength with the minimum of material.

Everywhere the inner structure of the femur agrees closely with that required by the static conditions imposed upon the bone by the preponderant load on the femur-head. In running the factor of safety of the normal femur analyzed is a minimum at the narrowest part of the neck, where it is 5.71 for compression: for tension, the factor of safety at this section is 5.68. These factors of safety depend upon the maximum strength of bone as determined by tests which give the ultimate strength of bone in compression at 24,000 pounds, and in tension 17,700 pounds per square inch. The factor of safety in the middle third, which is most exposed to violence, is somewhat greater than in the neck of the femur, being between 5.98 and 7.68, with an average value of 6.60. These factors of safety are based upon the maximum stresses that occur normally, which is assumed to be when running.

The factor of safety of the femur as a single structural element of the skeleton is determined by the strength of the weakest section with respect to the normal maximum stresses at that section. The weakest section of the femur with respect to the maximum stresses that occur at the various sections is in the narrowest part of the neck of the femur, about 2 inches from the head measured along the axis of the bone. At this section, which, therefore, determines the factor of safety of the entire femur, the factor of safety for the stresses due to running is 5.68; for the stresses due to walking the factor of safety is 11.36 and for the standing position it is 30.30.

The analysis of strength as outlined agrees with the actual breaking strengths of femurs made by Messerer, for the loads applied to the femur-head in the same direction as in life. The analysis is also in agreement with Messerer's breaking-tests on femurs by applying loads to the middle of the bone, which is supported at both ends.

Compression tests were made of the compact bone of one of the femurs analyzed in this paper and the breaking strength was found to be 25,100 pounds per square inch.

Statistics of the location of fractures of the femur in two independent series of 241 and 152 fractures, as given by Hyde and

Hamilton, respectively, are shown to agree very closely with the proportionate distribution of fractures according to the laws of probability, as applied by the writer to the femur.

General conclusions

The evidence presented in this paper is believed to warrant the following conclusions:

1. The normal external form and internal architecture of the human femur results from an adaptation of form to the normal static demands, or normal function of this bone.

2. The proportions of the femur are everywhere such as to show a definite mathematical relationship between the body weight, and the internal structure of the bone: there is a definite relation between the structure and the stress at every point.

3. Spongy bone is homogeneous with compact bone as a structural material and differs from it mechanically only in possessing smaller strength approximately in proportion to its density as compared with compact bone.

4. The femur has a factor of safety of 5.68 for the stresses due to running, 11.36 for the stresses due to walking, and 30.30 for the stresses due to standing. The weakest section for resisting the stresses due to loads on the femur-head is in the neck of this bone.

5. The structure of the femur is based upon the mathematical requirements of mechanics and the inner architecture is such as to produce great strength with a small amount of material and the disposition of the material at all points corresponds to the stress requirements at those points.

6. The general law of bone, the adaptation of form to function, holds true mathematically and mechanically in the normal human femur, and therefore for all other normal human bones.

Special conclusions

1. A foundation is laid for the study and mechanical analysis of the spongy bone entering into the structure of other parts of the skeleton, by the application of the principle that spongy

bone and compact bone are homogeneous materials and differ chiefly in strength approximately in proportion to their densities.

2. The thickness and closeness of spacing of trabeculae in bone vary directly with the intensity of the stresses transmitted by them.

Applications

If the mechanical structure of bone is correctly understood, the repair of fractures and the general treatment of bone diseases and deformities may be handled with greater efficiency and the proper prophylactic measures against deformity may be undertaken with greater hope of success.

The theory of the functional form of bone proposed by Wolff and also by Roux ('81) with that of the functional pathogenesis of deformity, though long supported by abundant clinical evidence and practically applied by many surgeons and orthopedists as a working basis in the treatment of deformities, has been the subject of so much controversy that much confusion has arisen as to the value of the theory in every-day practice.

The mathematical demonstration of the relation between the form and the function of bone under normal conditions as presented in this paper is believed to place this theory upon a sound foundation. The close adaptation of the structure of normal bone to its function leads logically to the conclusion that continued deviation from the normal static conditions to which a bone is subjected must be followed by a structural adaptation to meet the changed conditions (altered function).

Whether the persistent altered static (mechanical) conditions in the bone be due to fracture, bone disease, paralysis followed by postural changes, or other causes, the fundamental mechanical principles apply with equal force: transformation of the inner structure of bone takes place, and the inner structure of the bone is altered with mathematical accuracy to conform to the new mechanical conditions usually with a high degree of economy.

Fractures unite without deformity when there is good coaptation because in such a case the original mechanical conditions are restored and there is no need for excess bony material for resisting the normal stresses. However, if co-aptation is not secured and there is a gap between the fractured ends of the bone, union usually takes place with more or less shortening and the gap is bridged in the most economical manner that the altered positions of the parts of the bone will allow. The increased amount of material is required because of the greater stresses produced at the fracture.

In diseased bone the gradual weakening of the bone is followed by changes in form in accordance with mechanical laws: wherever the stresses in the bone exceed the strength of the bone distortion occurs until equilibrium of these forces is attained. It is clear that the essential in such cases to prevent deformity is by relieving the overloaded structure as completely as possible of all loads.

Postural variations from the normal produce increased stresses in certain regions and in others decreased stresses; if persistent, such variations will produce corresponding changes in the inner structure of the affected bones. In many instances there results a progressive increase of deformity until a condition of equilibrium is reached.

Whenever, from any cause a persistent change occurs in the manner in which loads are transmitted to the various parts of the skeleton an adaptive change must occur in the inner architecture of the bones in which these altered conditions exist. In the same manner that the application of this principle explains the production of deformity, it may be used to explain the cure of deformity. The proper mechanical means of imposing new mechanical conditions by which the original structure of bone may be restored is by the use of braces, jackets, or other suitable means which over-correct the deformity, and reverse the transformation process.

These results, so commonly secured by the orthopedic surgeon, depend upon the mathematically exact adaptation of the living bone to the mechanical conditions imposed persistently upon it by whatever cause or causes, and the necessary structural re-arrangements are usually attained with great economy of material.

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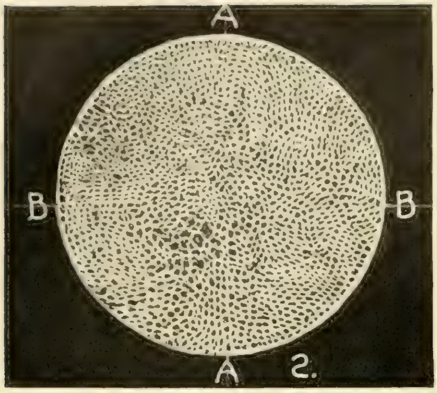
PLATES 1–5.

Plates 1–5 are serial transverse sections of the right femur of the same subject whose left femur is shown in longitudinal frontal section in figures 21, 24 and 25 and in outline in figures 14, 18, 19 and 19 *a*. Figures 14, 16, 17, 18, 19, 19 *a*, 20 and 27 and tables 5 and 6 are based upon the combined mechanical analysis of both these femurs; and the numbers of the sections in Plates 1–5 correspond in position to the similarly numbered lines in these figures and tables. Full details of the methods followed in the preparation and study of these sections are given in Part III under 'Analysis of the mechanical properties of the femur,' p. 227. (All sections are natural size.)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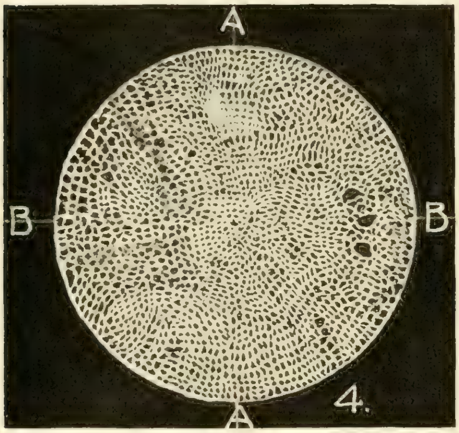
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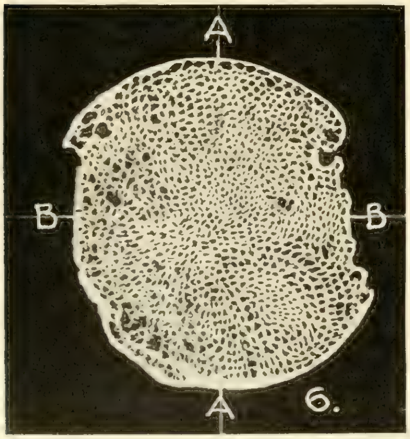
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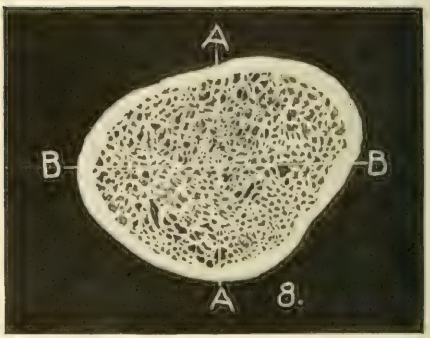
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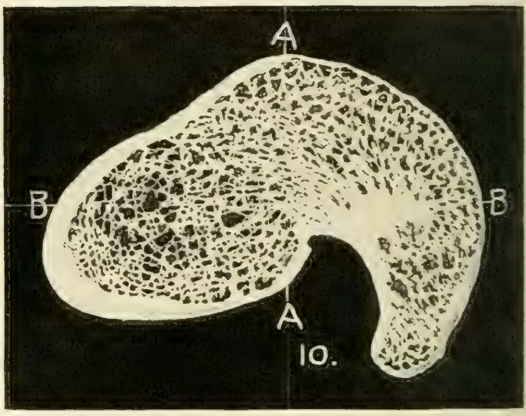
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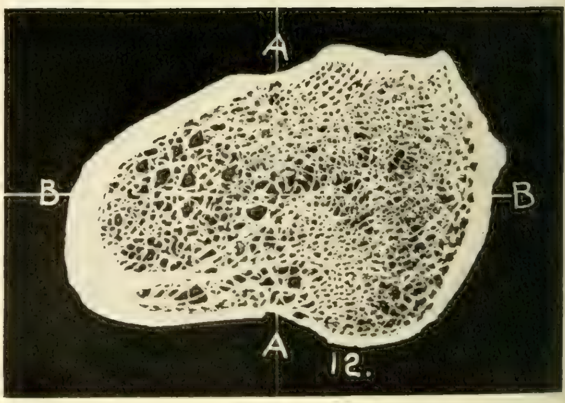
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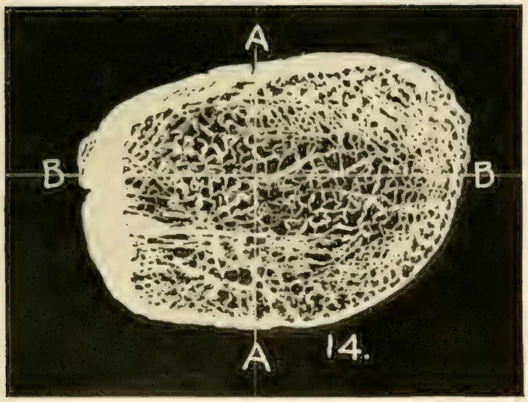

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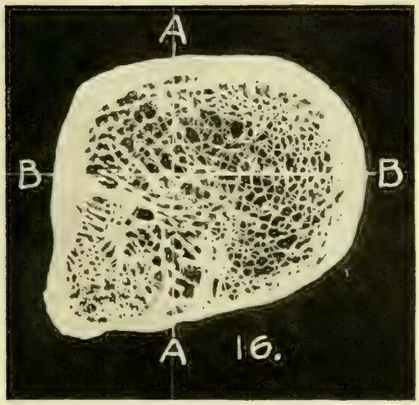

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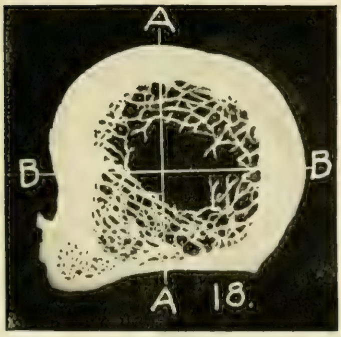

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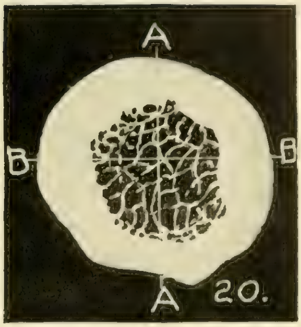

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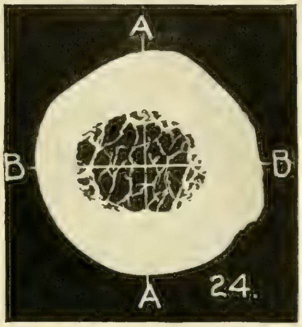

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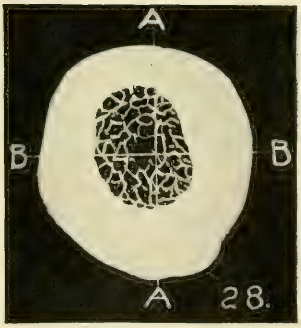

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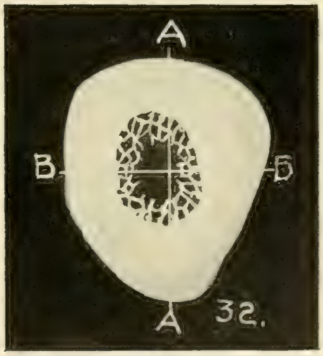

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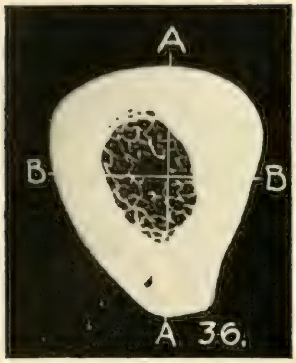

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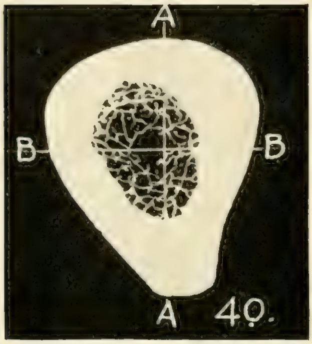

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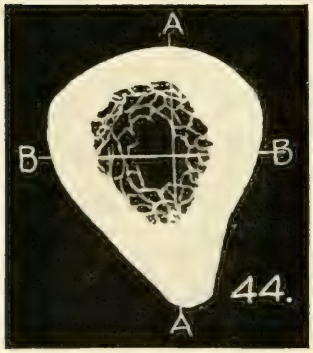

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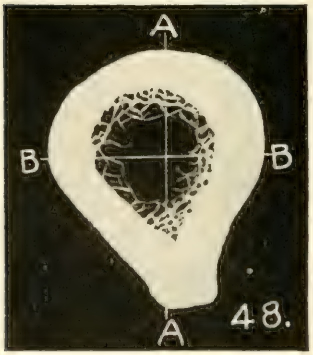

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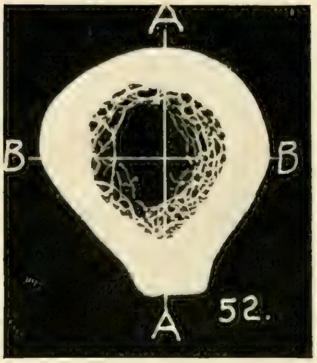

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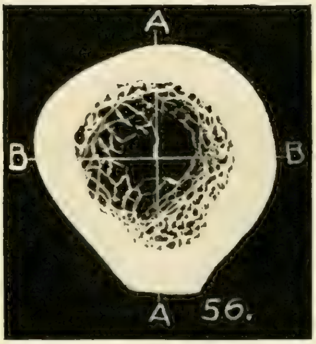

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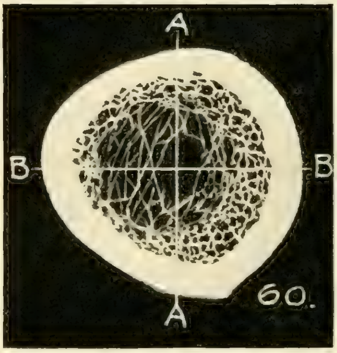

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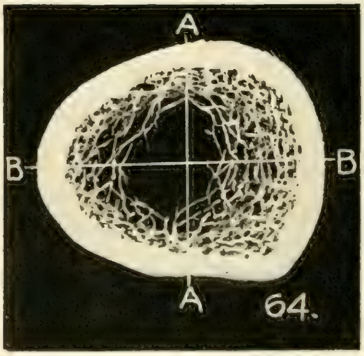

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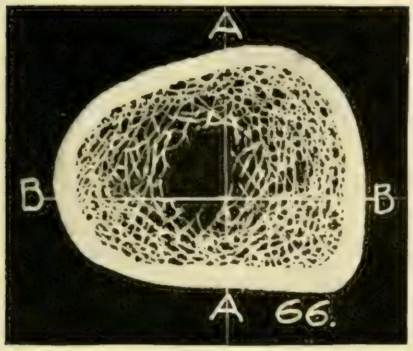

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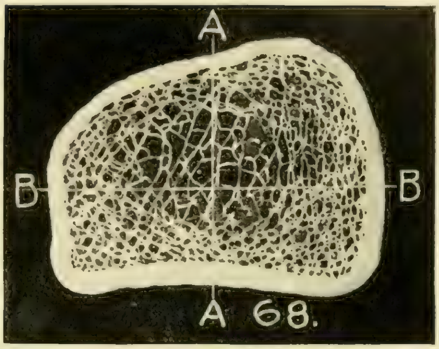

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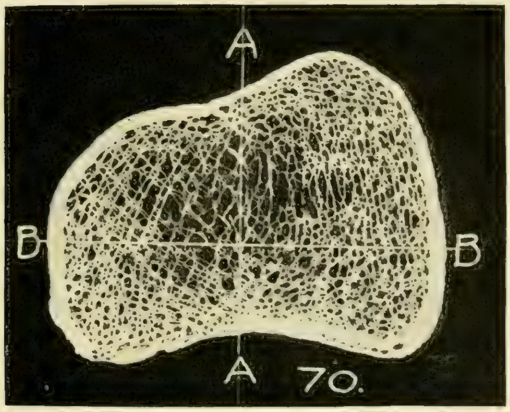

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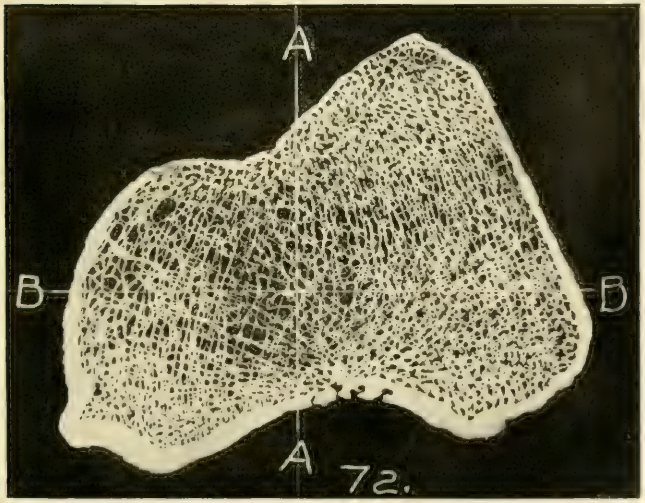

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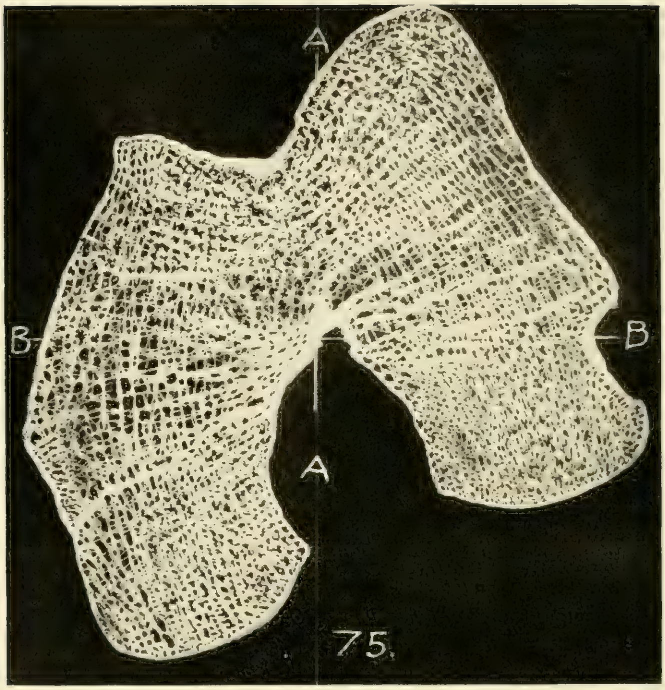
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THE DEVELOPMENT OF THE SCALA TYMPANI, SCALA VESTIBULI AND PERIOTICULAR CISTERN IN THE HUMAN EMBRYO

GEORGE L. STREETER

Department of Embryology, Carnegie Institution of Washington, Johns Hopkins Medical School, Baltimore, Maryland

NINE FIGURES

The study of the development of the large walled-off connective tissue spaces that surround the membranous labyrinth is particularly interesting in that it shows that they have a very definite morphological individuality. It is evident at least that they are not to be considered as insignificant accessories that merely fill in the waste intervals between the membranous labyrinth and the surrounding cartilage or bone. On the contrary, they have characteristics which are in many respects as definite and constant as those of the ossicles themselves. The individuality of these spaces in all respects is most marked. They make their appearance at a definite stage in the development of the embryo; they are formed at definite places; they pass through a series of definite histogenetic processes; they spread in a definite order and manner and eventually they attain a definite form and structure. The general morphology and relations of these spaces during their developmental period will be described in the following paper, and the opportunity will be taken to point out in the course of the description some of these individualistic features.

Instead of designating the large spaces surrounding the membranous labyrinth as perilymphatic spaces, as has been the general custom since the time of Breschet 1833, they will here be spoken of as perioticular or periotic spaces. The use of the term 'periotic' avoids the confusion arising from the incorporation of the word 'lymphatic' in the terminology. The present tendency is to restrict the use of the word 'lymphatic' to the lymphatic vascular system and its associated structures, with which these particular spaces have no known connection, either in their origin or in their ultimate relations.[1] We shall therefore speak of a periotic connective tissue that everywhere surrounds the epithelial portion of the labyrinth. This connective tissue includes, in part the fine-meshed periotic reticulum, and in part the large walled-off perioticular spaces to which belong the vestibular cistern, the scala vestibuli and the scala tympani with whose development we are primarily concerned.

MATERIAL AND METHODS

The observations that are recorded in this paper are all based on human embryos and cover the period included between embryos 35 mm. and 130 mm. CR length, which is approximately equivalent to the period between the ninth and sixteenth week of fetal life.

To facilitate the determination of the form and relations of the spaces, wax-plate models of the membranous labyrinth and the surrounding spaces were reconstructed after the Born method. Advantage was taken of the improvements in the method recently devised by Lewis 1915.[2] The serial sections were photographed at a suitable enlargement on bromide paper. By means of a preliminary model of the membranous labyrinth, the necessary reconstruction lines were established and inscribed on the bromide prints. From these prints then the membranous labyrinth and the perioticular spaces were traced on wax-plates. After cutting out from the plates the areas corresponding to these structures, the plates were piled and the resultant cavities were filled with plaster of Paris. The wax was finally melted off and there was left then a permanent plaster cast of the objects desired at a definite enlargement. Views of these models are shown in figures 4 to 9.

In outlining the periotic spaces it was found necessary to make an arbitrary rule as to how much should be included in the model. The smaller spaces of the reticulum that surrounds the main cavities can be seen coalescing to form larger spaces and these in turn coalesce with the main cavity as it advances into new territory. Thus in a given section there is a considerable range in the size and completeness of the spaces. The main spaces and the larger adjacent ones that communicate with them are outlined by a membrane-like border. This characteristic was utilized as the guide for determining which spaces to admit into the model; only those possessing a more or less complete border of this kind were included.

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[1] Sabin, F. R. Harvey Society Address. Science, vol. 44, 1916, p. 145.

[2] Lewis, W. H. The use of guide planes and plaster of Paris for reconstructions from serial sections. Anat. Rec., vol. 9, 1915.

HISTOGENESIS OF THE PERIOTIC RETICULUM

Although this communication is more concerned with the process of conversion of the periotic reticular tissue into the larger walled-off spaces, yet for the purpose of completeness a brief survey will be taken of the earlier history of this tissue and the nature of its histogenesis.

The tissue in which the perioticular spaces develop is derived from the condensed mesenchyme that establishes itself as an encapsulating mass around the otic vesicle in embryos between 4 mm. and 10 mm. long. This condensed mesenchyme is subsequently differentiated into the cartilagenous capsule that completely invests the epithelial labyrinth excepting for the three openings that persist in the adult as the internal auditory meatus, the aqueaductus cochleae and the aquaeductus vestibuli, which openings are present in the very earliest stages.

Originally the cartilagenous capsule abuts directly against the epithelial wall of the labyrinth. In embryos about 14 mm. long, however, the cartilage-forming tissue in the immediate neighborhood of the epithelium undergoes a dedifferentiation, so that an area is established all around the membranous labyrinth, and conforming to it in shape, that is less like cartilage and more like embryonic connective tissue. It is this that constitutes the foundation for the open-meshed periotic reticulum which in embryos 30 mm. long everywhere bridges the space existing between the membranous labyrinth and the surrounding cartilage. The membrana propria that supports the epithelial part of the labyrinth and the perichondrium lining the cartilage are both derived from this periotic reticulum. It is also a modification of the meshes of this same reticulum that results in the formation of the perioticular spaces in a manner that will now be outlined.

Unmodified periotic reticulum is characterized by a rather uniform narrow mesh. The essential change which it undergoes in the process of space formation consists in the disappearance of some of the trabeculae of the mesh followed by the coalescence of the corresponding adjacent spaces. The trabeculae consist of the protoplasmic processes of the constituent cells of the reticulum and their disappearance is probably to be explained, not by a dissolution or liquefaction of these cell-processes but by an alteration in their form. It apparently is the result of an active motility of the cell protoplasm involving the successive detachment and retraction of the trabeculae. When a trabecula becomes detached it gradually retracts and adapts itself to the formation of a larger space, reshaping itself either as a smooth border or as a constituent part of another trabecula. As spaces become larger they require longer trabeculae, and as trabeculae become longer they also tend to become thicker.

The differentiation of the margin of the periotic spaces constitutes the final feature in their maturation. During the period in which the enlargement of an individual space is actively going on, the margins of the main cavity consist of smooth delicate strands of nucleated protoplasm that resemble the trabeculae between the large reticular spaces. These linear margins are interrupted here and there by openings into adjacent spaces. They tend, however, to form a continuous line that definitely marks off the space from the adjacent reticulum. As the space becomes more mature, the membrane-like border becomes thicker until it reaches a state that will probably not admit of any further opening-up for the coalescence of additional spaces. Any further growth is thereafter limited to simple distention of the wall of the space with the consequent adjustment of its constituent cells. In its final form the margin of the space roughly resembles an endothelial membrane. Immediately lining the space is a thin membrane with flattened nuclei which is supported underneath by a thin coat of nucleated protoplasm that has the form of fibrous connective tissue. The former, judging only from its final appearance, could be designated as endothelium, thus making a distinction between it and the underlying tissue. In its histogenesis, however, it differs in no way from the rest of the wall and the difference that exists later seems to be merely the result of its adaptation to the existing physical conditions. Its early behavior is entirely different from that of vascular endothelium. Therefore if one uses the term endothelium for its designation this must be done with a considerable amount of reservation.

These phenomena can be particularly well studied in the scalae while they are in the process of spreading and enlarging. As we shall see, the scalae are more mature in their proximal portions and are progressively less mature as one approaches the apex of the cochlea. Thus any one specimen shows several stages in the development. Typical views showing some of the steps in this process are represented in figures 1 to 3. Figure 1 represents a section through the second turn of the cochlea in a human fetus 130 mm. CR length (Carnegie Collection, No. 1018). It shows the topography of the cochlear duct and the general character of the perioticular spaces that are developing along its inner margins. The upper one or scala vestibuli is in a more mature condition. The lower one or scala tympani is less mature and along its peripheral (right) margin, it is in the act of spreading so as to underlie, as it eventually will do, the future basilar membrane. The scala tympani finally reaches the peripheral margin of the cochlear duct, and it does this by the coalescence of the enlarging reticular spaces which become incorporated with the main cavity of the scala.

This area is a particularly good one for studying the histogenesis of these spaces. It is shown under higher magnification in figure 2, which is a detail of the same section. By comparing this figure with figure 1, the exact location can be readily made out. That portion of the cochlear duct that is to form the organ of Corti can be recognized by the characteristic form and grouping of its cells. A portion of the main cavity of the scala tympani is indicated and to the right of this are a few enlarged reticular spaces that are uniting with each other subjacent to the organ of Corti and the basilar membrane. These will in the end become part of the main space. They are here just in the process of coalescence, the histological features of which procedure are well illustrated in this figure. The trabeculae are stretched out in long strands and in many cases are detached and project into the spaces as free ends. The detached trabeculae are seen in different degrees of retraction as their constituent protoplasm reshapes itself in adaptation to the new boundaries. It is only at the margins of the larger spaces that the cell-processes exhibit the characteristic flattened appearance,

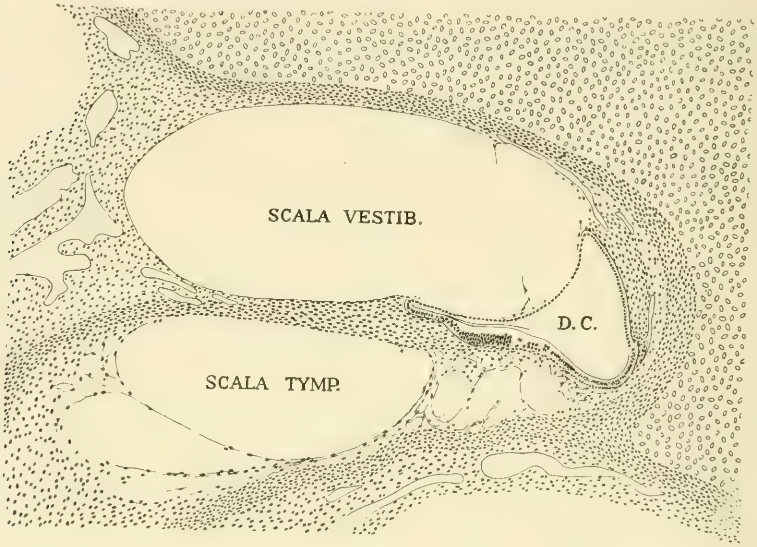


Fig. 1 Section through the second turn of the cochlea in a human fetus 130 mm. CR length (Carnegie Collection, No. 1018). Enlarged 60 diameters. This section shows the topography of the cochlear duct and the general character of the periotic spaces that are developing along its inner margin. Details of this same section as seen under higher magnification are shown in figures 2 and 3.

which is the first indication of the formation of the marginal membrane. The residual undifferentiated reticulum that does not enter into the direct formation of the larger spaces constitutes the tissue from which is derived the adventitial coat of the completed scala.

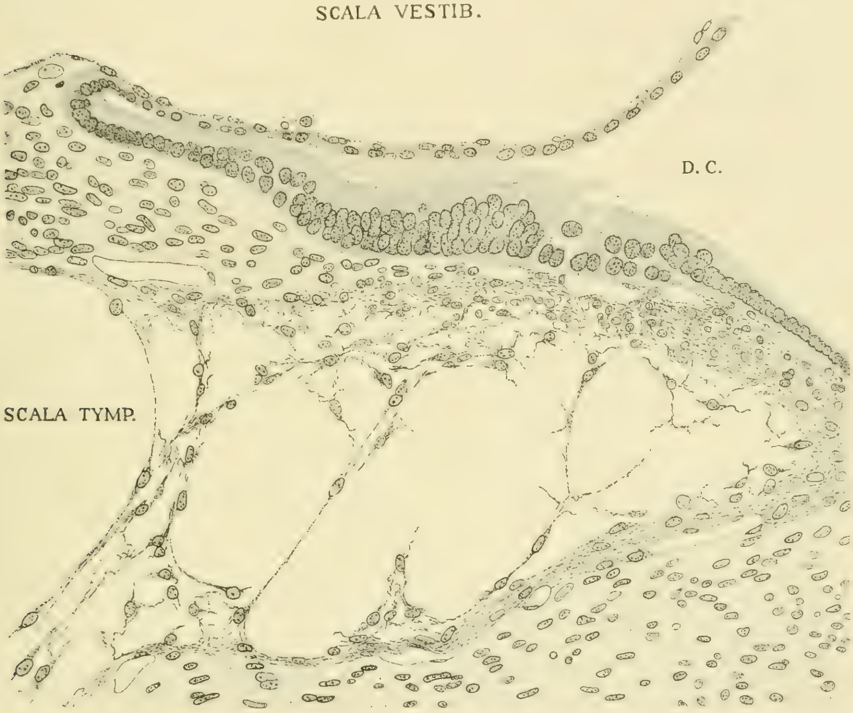


Fig. 2 Detail of the section shown in figure 1, enlarged 278 diameters. This figure shows the part of the cochlear duct that is to form the organ of Corti, and the adjacent tissue that becomes incorporated in the basilar membrane. Below this is the periotic reticulum whose spaces are in the process of enlarging. By repeated coalescence these spaces finally unite with the large space that constitutes the scala tympani. This figure shows the histological appearance of the reticulum where the formation of tissue spaces is in active operation.

The appearance of the marginal membrane as seen in a more mature space is shown in figure 3, being a detail of the margin of the same scala vestibuli that is shown in figure 1. Here we have a firm membrane that forms a complete barrier between the periotic reticulum and the lumen of the scala. After reaching this degree of development there is no evidence of any further coalescence of the surrounding reticular spaces with the main cavity. The membrane itself as seen in cross section consists of rather compact nucleated strands of protoplasm, which cannot as yet be separated into the so-called endothelial coat and the supporting fibrous coat. However, a comparison of the coagulated elements of the fluid seen in the reticular spaces with those seen in the scala would indicate a difference between the two and therefore it is probable that the membrane is already partially impervious.

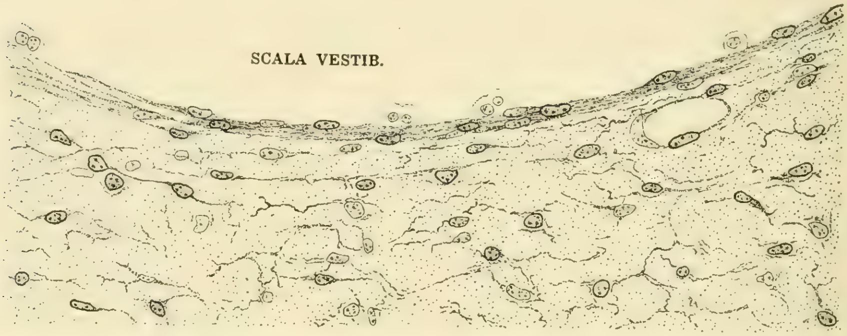


Fig. 3 Detail of the section shown in figure 1, enlarged 400 diameters. It shows the character of the margin of the scala vestibuli in a more mature condition. The scala vestibuli is inclosed by a membrane consisting of the cells that had previously constituted the reticulum occupying this area and which have been modified in form in adaptation to the formation of this large tissue space, closing it off from the surrounding tissue.

DEVELOPMENT OF THE PERIOTIC CISTERN OF THE VESTIBULE

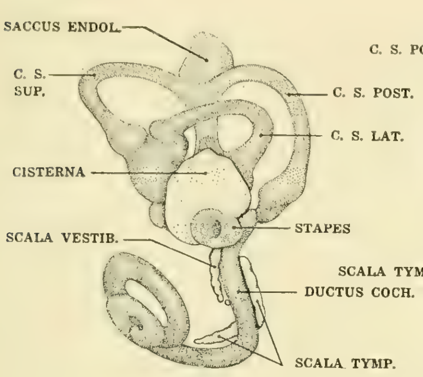
Aside from the scala vestibuli and the scala tympani, the largest of the periotic spaces is the large reservoir situated between the tympanic wall of the bony vestibuli with its articulated stapes, and the vestibular chambers of the membraneous labyrinth. This is the spatium perilymphaticum vestibuli (BNA) or the cysterna perilymphatica (Retzius). In order to eliminate the word lymphatic from the terminology it will be designated here as the Cisterna periotica vestibuli or less formally the periotic cistern. In this manner the descriptive term introduced by Retzius is retained.

Before there is any trace of the scalae the initial steps in the formation of the cistern can be seen. This is well illustrated in an embryo 35 mm. long (Carnegie Collection, No. 199). This particular embryo is cut into a sagittal series and the sections on slides 53 and 54 show the periotic cistern in its most rudimentary form. It consists of an area of reticulum bounded by the utricle, saccule, ductus reuniens, the proximal end of the cochlear duct and the ampulla of the posterior canal. The reticulum here is of the type seen along the semicircular canals in considerably older embryos. Whereas the reticulum elsewhere in this 35 mm. embryo presents a uniformly narrow mesh that is interrupted only by the numerous capillaries branching through it, this particular field gives the appearance of spaces which are more open and which are irregular both in shape and in size. From the very first the increase in the size of the mesh seems to be attained by the detachment and retraction of its constituent protoplasmic bridges, thereby allowing adjacent spaces to unite in the formation of composite larger spaces. Thus in the above section a few irregular protoplasmic free-ends are seen still projecting into the newly enlarged spaces. The area of this rudimentary periotic cistern is as yet very small and merges indefinitely into the adjoining reticulum. It is not until we come to fetuses about 40 mm. long that it develops spaces of any considerable size, and it is not until we come to fetuses about 50 mm. long that we find a single large space with walls that are definitely outlined so that it can be satisfactorily modelled.

In a fetus 43 mm. long (Carnegie Collection, No. 886), the spaces forming the rudimentary cistern stand out much more definitely than is the case in the 35 mm. embryo that has just been referred to. There is now just opposite the stapes one space which is much larger than the adjoining spaces. On part of its margin the protoplasmic bridges are stretched along so as to form a smoothly curved continuous boundary. This boundary is defective in some portions and at such places the space merges with the adjoining secondary spaces. Within the space are some faintly refractive branching threads of coagulated plasma. The scala vestibuli is not yet laid down and the scala tympani is only represented by a moderate widening of the meshes of the reticulum in the neighborhood of the fenestra cochleae (rotundum), along the basal border of the first turn of the cochlear duct.

In fetuses 50 mm. long the outlines of the cistern become very distinct due to the marked increase in the size of its main cavity and to the more definite membrane at its junction with the rest of the reticulum. Its form and relations are shown in figures 4 and 5. They represent a median and a lateral view of a waxplate reconstruction of this region in a human fetus 50 mm. long (Carnegie Collection, No. 84). Only the main cavity is shown in the model. At certain places around its borders the meshes of the reticulum are uniting into larger spaces and these in turn are taken up by the main cavity as it advances into the new territory. These smaller incomplete spaces were omitted in constructing the plates of the model.

It will be seen then from figure 4 and 5 that the periotic cistern in 50 mm. embryos consists of a flattened rounded bursa-like cavity that intervenes between the stapes and the lateral surface of the saccule and adjoining utricle. It extends forward to the ampulla of the lateral canal and upward to the beginning of the crus commune. Posteriorly it crowds backward against the ductus reuniens filling in the space between the utricle, saccule and the proximal end of the cochlear duct. Both on its median and lateral surfaces there is no further opportunity for expansion except as the vestibule itself enlarges. The delicate membrane-like wall of the cistern hugs closely against the parts of the membranous labyrinth on the one side and the tympanic wall of the cartilagenous vestibule on the other, being separated from them only by a thin layer of the original reticulum. Along the dorsal margin of the cistern, however, there is room for expansion and the reticulum in this region shows enlarging spaces in the process of uniting with the main cavity. On its ventral margin near the cochlea and extending along the apical surface of the latter there is a definite row of reticular spaces actively coalescing and constituting the beginning of the scala vestibuli. The scala tympani is already well started at this time, but its development is quite independent of the cistern. Within the cistern can be seen scattered clumps of faintly refractive granular threads of what seems to be a coagulated constituent of the plasma.



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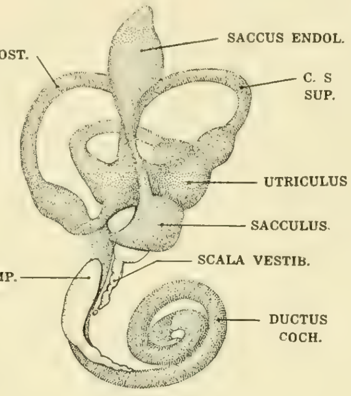

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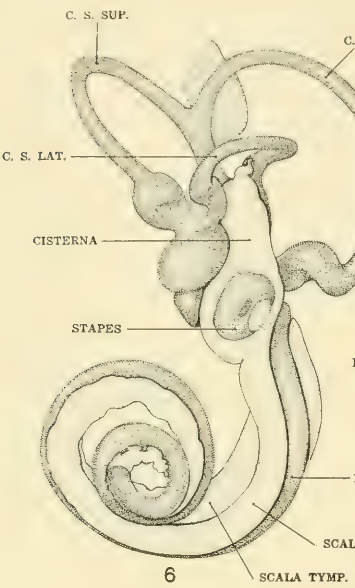
Fig. 4 The figures 4 to 9 represent a series of median and lateral views of wax-plate reconstructions of the membranous labyrinth and the surrounding perioticular tissue spaces, illustrating under the same scale of enlargement three typical stages in the development of these spaces. This figure shows a lateral view of a model reconstructed from a human fetus 50 mm. CR length (Carnegie Collection, No. 84). The scala vestibuli is in the first stage of its development and consists of a row of large reticular spaces which extend from the ventral margin of the cistern downward along the apical surface of the cochlear duct. The scala tympani is more advanced and shows more complete coalescence of its constituent spaces. Enlarged 9 diameters.

Fig. 5 Median view of the same model shown in figure 4. This view shows the topography of the scala tympani. Its large proximal end lies opposite the fenestra cochleae and corresponds to the focus at which its development originates. Distally it tapers off rapidly, where the spaces are smaller and their coalescence less complete. Enlarged 9 diameters.

The subsequent growth of the cistern is shown in figures 6 to 9. Figures 6 and 7 show respectively a median and lateral view of a wax-plate reconstruction of the membranous labyrinth and its perioticular spaces in a human fetus 85 mm. long (Carnegie Collection, No. 1400–30). The growth of the cistern here has kept pace with the increase in size of the labyrinth and maintains the same general relations as regards the stapes and the parts of the membranous labyrinth. The view of the cistern in figure 6 is an oblique one which would tend to mislead one as to its width. In reality it is relatively a little wider.



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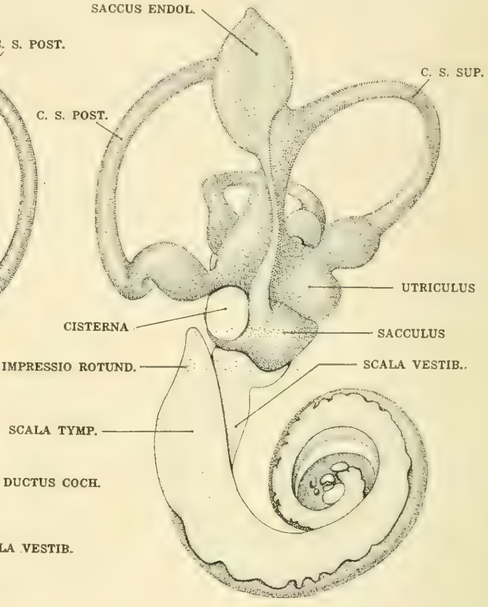

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Fig. 6 Lateral view of reconstruction of the left membranous labyrinth and the periotic spaces in a human fetus 85 mm. CR length (Carnegie Collection, No. 1400–30) enlarged 9 diameters. Although the greater part of the cistern abuts against the stapes it will be noted that it also is beginning to spread over the dorsal surface of the utricle and along the inner border of the lateral canal. The scala vestibuli communicates freely with the cistern and extends downward along the apical surface of the cochlear duct throughout nearly two turns, showing the characteristic sacculated appearance near its tip where the coalescence of the spaces is less complete.

Fig. 7 Median view of same model shown in figure 6. The oval indentation in the proximal end of the scala tympani corresponds to the fenestra cochleae. This space extends along the cochlear duct about the same distance as the scala vestibuli; the two however do not communicate with each other as yet. The peripheral border of the scala tympani is characterized by sacculations corresponding to spaces that are coalescing with the main space. This indicates the direction of the growth of the scala at this time.

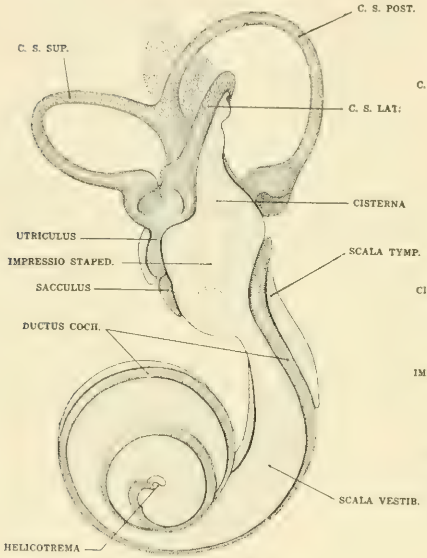
It has also extended upward on the dorsal surface of the utricle and is beginning to creep along the inner side of the posterior end of the lateral canal. Ventrally it communicates freely with the scala vestibuli which now extends well down along the cochlear duct.

The oldest stage studied is shown in figures 8 and 9. These show two views of a wax-plate reconstruction of these structures in a human fetus 130 mm. long (Carnegie Collection, No. 1018). At this time the periotic cistern has spread over the vestibular part of the membranous labyrinth, covering it nearly everywhere excepting at the macular portions where the nerves terminate. In figure 9 it can be seen that the mesial surface of the saccule is not covered; this lies closely against the wall of the cartilagenous vestibule. The uppermost division of the cistern situated between the crus commune and the ampulla of the posterior canal does not yet open into the general cavity. It has formed separately and owing to the position in which it lies its coalescence with the other parts of the cistern is retarded. Otherwise free communication exists between all divisions of the cistern.

DEVELOPMENT OF THE SCALA TYMPANI AND SCALA VESTIBULI

The scala vestibuli may be regarded as an extension downward of the cistern into the region of the cochlea and as such its growth starts from a focus opposite the fenestra vestibuli (ovale). The scala tympani in a similar way makes its first appearance opposite the fenestra cochleae. From these two foci the scalae extend gradually downward along the cochlear duct as two separate spaces which do not communicate with each other until they reach the tip of the duct, where there is finally developed a free opening between them known as the helicotrema.

In their formation they go through a series of histogenetic changes in essentially the same manner that has been followed in the case of the formation of the cistern. This as we shall see consists of the enlargement of the spaces of the periotic reticulum that originally occupies this region, the enlargement being a result of the disappearance of the protoplasmic bridges of the reticulum whereby adjacent spaces unite in the formation of composite larger spaces. This process continues until there is a single continuous space extending down along the cochlear duct representing each scala and at the margins of each of them there is developed a membranous arrangement of the reticular cells which completely walls off the space from the surrounding tissue. In these alterations in the reticular mesh and in the formation of the surrounding membrane there is an active change



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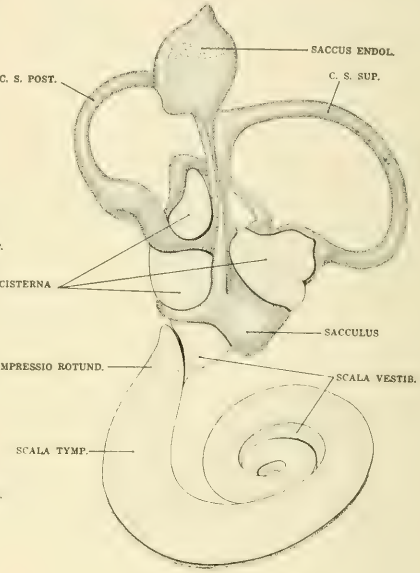

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Fig. 8 Lateral view of a wax-plate reconstruction of the left membranous labyrinth and the periotic spaces in a human fetus, 130 mm. CR length (Carnegie Collection, No. 1018) enlarged 9 diameters. The cartilagenous stapes was removed from this model and the oval impression that it makes on the cistern can be plainly seen. The cistern has spread over the top of the utricle and along the lateral canal. The scala vestibuli extends to the tip of the cochlear duct where it communicates with the scala tympani, thus forming the helicotrema.

Fig. 9 Median view of the same model shown in figure 8. The oval impression on the proximal end of the scala tympani corresponds to the fenestra cochleae. As yet there is no communication at this point between the scala tympani and the subarachnoid spaces corresponding to the aquaeductus cochleae. The spaces making up the cistern cover almost the whole of the utricle and saccule excepting the places at which the nerves enter and a small part of the medial surface near the attachment of the appendage.

in the form of the reticular cells which repeatedly adapt themselves to the new conditions. There is no evidence to indicate that any other cells take part in the formation of the scalae.

The first evidence of the formation of scalae is found in fetuses about 40 mm. long, which is a little later than the first appearance of the cistern. In a fetus 43 mm. CR length (Carnegie Collection, No. 886) along the proximal part of the cochlear duct on its basal surface, there is a distinct widening of the meshes of the periotic reticulum. This is the beginning of the scala tympani. On the opposite side of the cochlear duct where one would look for the scala vestibuli the periotic reticulum retains its primitive appearance characterized by a narrow and rather uniform mesh. Thus the scala tympani makes its appearance slightly in advance of the scala vestibuli, that is, if we regard the latter as distinct from the cistern.

In fetuses 50 mm. long both the scala tympani and the scala vestibuli can be plainly identified, although they are still very incomplete. A wax-plate reconstruction of them representing their form and their relation to the membranous labyrinth in a human fetus 50 mm. CR length (Carnegie Collection, No. 84) is shown in figures 4 and 5, being a median and a lateral view respectively. It will be seen that the scala tympani is larger and more advanced in its development than the scala vestibuli. The latter is in its earliest stage and consists of hardly more than a row of enlarged reticular spaces that extend downward from the cistern along the dorsal and apical surface of the cochlear duct.

The scala tympani consists of an elongated oval space lying along the basal surface of the proximal part of the cochlear duct, about corresponding to the proximal half of the first turn of the duct. In the main part it is a single space with a distinct margin separating it from the general periotic reticulum. In the more apical portion it tapers off into multiple incompletely united smaller spaces which actively coalesce as the process advances into the new territory along the duct. It is of interest to note that the most mature and the largest part of this scala, representing the focus at which it first appeared, is opposite the fenestra cochleae (rotundum), just as the cistern forms opposite the stapes and the fenestra vestibuli. The scala tympani always begins at the same place and extends downward along the cochlear duct, at first a little in advance of the scala vestibuli, but subsequently the latter catches up with it and the two reach the tip of the duct at about the same time.

It is well known that the proximal portions of the cochlear duct mature sooner than the distal portions. One might expect that the accompanying periotic spaces would correspond in their development to the maturity of the duct and therefore the proximal parts of the scalae would differentiate first. In other words the maturation of the cochlea proceeds as a wave from the proximal end to its tip involving all of its constituent structures as it passes along, including mesenchymal parts as well as epithelial. This conception might explain the direction of development of the scalae but it can hardly be applied to the cistern, the vestibular representative of the scala vestibuli. One cannot say that those portions of the membranous labyrinth lying opposite the focus of development of the cistern, that is, the lateral walls of the saccule and utricle mature in advance of the rest of the labyrinth. There is no indication that a wave of differentiation passes through the epithelial elements of the labyrinth in the same direction and synchronously with the extension of the cistern as it advances from its primary focus up on the roof of the utricle and over on its median surface. In the case of the cistern it seems much more likely that the point at which it first appears is determined by the position of the stapes, which is doubtless an expression of the physical relation that subsequently exists between the two. By analogy this would yield additional significance to the relation existing between the fenestra cochleae and the point of beginning development of the scala tympani.

The form and relations of the scalae in fetuses between twelve and thirteen weeks old are shown in figures 6 and 7. These figures show median and lateral views of a wax-plate reconstruction of the membranous labyrinth and the surrounding periotic-ular spaces in a human fetus 85 mm. CR length (Carnegie Collection, No. 1400–30). Attention has already been directed to these figures in the description previously given of the cistern. The scala vestibuli can be seen in figure 6. Above, it opens freely into the cistern and extends downward along the apical side of the duct as a single main space, possessing a rather uniform diameter. It extends along the first two turns of the duct, gradually tapering off and showing a less mature character in its distal portions. Along the second turn of the duct the spaces are incompletely fused and the contour becomes correspondingly irregular. As a rule the peripheral margin of the scala seems less mature and more irregular than the central margin. The scala vestibuli does not connect with the scala tympani at any point as yet. The two are separated in the first place by the cochlear duct and then more centrally by a framework of connective tissue in which are the radiating bundles of the cochlear nerve with the nodes of ganglion cells that form the spiral ganglion. These latter structures are not shown in the model, they occupy however the V-shaped groove seen between the two scalae.

The scala tympani, as can be seen in figure 7, extends downward on the basal side of the cochlear duct along its first two turns. This corresponds to about the same linear dimension as that of the scala vestibuli. In its proximal portion it shows a greater area in cross section than the latter, but further toward the apical region it is about the same size and in some places it is even smaller. The peripheral margin of the scala tympani is distinctly more irregular than the central margin. This irregularity is due to spaces along the margin that are actively coalescing with the main space, but in which the fusion is not yet complete. The irregularity of this margin is thus an indication of the direction of the expansion of the scala. As the diameter of the whole cochlear mass increases it is evident that the main growth of the scala must radiate outward in a peripheral direction. This is accomplished by the continual assimilation of new reticular spaces along this margin. At the proximal end of the scala tympani can be seen an oval depression which corresponds to the fenestra cochleae (rotundum) and with which it stands in intimate relation.

In fetuses about sixteen weeks old the form and relations of the scalae have nearly attained the adult conditions and this represents the oldest stage studied in connection with the present paper. The conditions found at that time are shown in figures 8 and 9 which present median and lateral views of a wax-plate model of a human fetus 130 mm. CR length (Carnegie Collection, No. 1018). On comparing the scala tympani and scala vestibuli as seen in these figures with those in figures 6 and 7 it will be seen that they are larger in cross section and more nearly cover in the cochlear duct. Furthermore they now extend to the extreme tip of the duct and communicate with each other across its central margin thus forming a helicotrema. It will be noted that now even as far as the tip of the cochlea each of the scalae consists of a continuous principal space. They are however more mature and larger in their proximal portions. Along the first turn of the cochlear duct they are walled off by a smooth membranous margin which separates them from the adjacent reticular tissue. The spaces of the latter do not seem to be taking any further part in the process of enlargement of the scalae. Along the second turn of the cochlear duct, a section of which was shown in figure 1, the coalscence of reticular spaces with each other and with the scalae is still in active operation. This produces a greater irregularity of the scalae than is shown in the model. The subsidiary spaces are shown as a solid mass, the slender clefts separating them are not represented. The nearer we approach the tip of the duct the more immature are the scalae until the condition is reached where the membrane-like margin is quite incomplete and the spaces merge irregularly with the surrounding reticulum. Thus a single specimen if studied in its different parts shows several stages in this interesting process of the formation and growth of the scalae.

PERIOTIC SPACES OF THE SEMICIRCULAR CANALS

From the descriptions given of the adult the reticulum along the canals never develops a single continuous wide periotic space like that of the cistern and the two scalae. There always remain a few trabeculae such as are seen in the cistern and scalae in their earlier stages, and these constitute partitions which subdivide the space and give it the appearance of a series of separate spaces extending along the inner margins of the canals. Although these spaces along the canals are incomplete as compared with the cistern and scalae, they are however entirely analogous with them in their formation.

The space along the lateral canal is the largest. Its posterior end exists as a continuation of the cistern. This can be seen in the lateral view of the model shown in figure 8 where the cistern extends for a considerable distance along the inner border of the lateral canal. Along the other two canals of the same specimen (130 mm., CR length) the reticulum has commenced the process of space-formation, but complete channels are not yet established.

COMMUNICATION OF THE PERIOTICULAR SPACES WITH THE ARACHANOID SPACES

The relation of the scala tympani and scala vestibuli to the subarachnoid spaces surrounding the hind-brain is of considerable interest both on account of the possibility of their functional relationship and on account of the similarity that exists in their development. For a satisfactory investigation of the establishment and the character of the communications that are formed between these two allied systems of tissue-spaces one should resort to other methods than those used in the present study, and furthermore one should examine older fetuses than those described here. Certain observations, however, were made in the course of the above investigation that bear a relation to these matters, and they will be briefly outlined here. In the first place the histological picture of the periotic reticulum is essentially the same as the early stages of the pia-arachnoidal tissue, that invests the central nervous system. The enlargement of the meshes of the latter and the formation of the subarachnoid spaces and the arachnoid cistern, as has been recently described by Weed,[3] correspond exactly with the appearances seen in the histogenesis of the perioticular spaces in the ear. The perioticular spaces are not however extensions of the arachnoid spaces that have invaded the cavity of the cartilagenous labyrinth. If this were so we should find them first appearing among the rootlets of the vestibular and cochlear nerves along which the subarachnoid space extends for some little distance. Instead, they begin at points where there can be no connection with the arachnoid tissue and their direction of growth is quite independent of it. The perioticular spaces may be analogous to the arachnoid spaces, but they are not identical with them, nor are they an extension of them.

According to the descriptions of the adult anatomy of the ear a communication becomes established between the scala tympani and the subarachnoid space near the fenestra cochleae, the so-called aquaeductus cochleae. Vague and conflicting statements are also made concerning a communication through the internal auditory meatus connecting the arachnoid spaces with the scalae. Such communications must be established quite late. In the oldest fetus examined, 130 mm. CR length, they do not yet exist. As to the latter communication it can be seen that the arachnoid spaces extend peripherally through the internal auditory meatus along the trunk of the acoustic nerve-complex and slender pockets and clefts from them extend along the larger bundles of the cochlear nerve; they terminate, however, before reaching the margins of the scalae, and there is no evidence at this stage that there is ever to be a communication between them and the scalae. As to the aquaeductus cochleae in the 130 mm. fetus it can be plainly seen that it is already forming as a derivative of the arachnoid spaces although the communication with the scala tympani is not yet established. The arachnoid spaces invest the glossopharyngeal nerve and extend down along its trunk and pass closely and directly posterior to the region of the fenestra cochleae (rotundum). A thin-walled tubular pouch projects from these spaces leaving the nerve trunk and extending obliquely toward the scala tympani in a direction that would meet it just distal to the fenestral impression on its basal surface. This fundament of the aquae ductus cochleae is present in fetuses 85 mm. CR length, but is longer in the 130 mm. fetus where it nearly reaches the scala tympani. The communication must be established soon after this.

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[3] Weed, L. H. The development of the cerebro-spinal spaces in pig and in man. Contributions to Embryology, vol. 5, No. 14, Publications of Carnegie Inst. of Wash., No. 225, 1917.

SUMMARY

The earliest histological evidence of the formation of the perioticular spaces occurs near the stapes, in the reticulum lying between the sacculus and the fenestra vestibuli, where in embryos between 30 mm. and 40 mm. long it can be seen that its meshes are becoming irregular and its spaces are beginning to coalesce. This constitutes the rudiment of the vestibular cistern. It makes its appearance before there is any trace of the scalae, but it is not until the fetus becomes about 50 mm. long that the cistern is definitely outlined and clearly differentiated from the adjoining reticulum.

After the cistern, the scala tympani is the next space to become established. It can be recognized as a moderate widening of the meshes of the reticulum in the region of the fenestra cochleae in fetuses 43 mm. long, along the basal border of the first turn of the cochlear duct. The scala vestibuli, as can be seen in fetuses 50 mm. long, develops as an extension downward of the cistern along the apical border of the cochlear duct. Starting from these definite foci these three spaces spread into their destined territory absorbing as they go the enlarging reticular spaces of the invaded region by a process of space-coalescence. In fetuses 85 mm. long the two scalae extend downward along the cochlear duct to its last turn as two separate spaces which do not communicate with each other. When they reach the tip of the duct, which occurs in fetuses about 130 mm., crown-rump length, a free opening is developed between them and this represents the helicotrema.

The perioticular spaces are analogous in their development to the pia-arachnoidal spaces; they are not however extensions of them that have invaded the cavity of the cartilagenous labyrinth. They begin at points where there can be no connection with the arachnoidal tissue and their direction of growth is quite independent of it. The communication that is found in the adult between the scala tympani and the subarachnoid space in the neighborhood of the fenestra cochleae, the so-called aquaeductus cochleae, is established quite late. In fetuses 85 mm. CR length it exists as a tubular pouch projecting from the subarachnoid spaces along the glossopharyngeal nerve toward the scala tympani. In the 130 mm. fetus, the oldest examined, this pouch is longer and nearly reaches the scala. The communication must be established soon after this.

Similar projections from the subarachnoid spaces at the internal auditory meatus extend as perineural clefts along the trunk and branches of the acoustic nerve. No actual communications were seen between these spaces and the two scalae.

EFFECTS OF INANITION AND REFEEDING UPON THE GROWTH AND STRUCTURE OF THE HYPOPHYSIS IN THE ALBINO RAT

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On account of the intimate relations usually assumed to exist between the hypophysis and the general growth process of the body, the effects of inanition and refeeding upon this organ have unusual interest and importance. Few observations upon these effects are recorded in the literature. In previous papers (Jackson '15 a; '15 b) it was shown that, in young rats held at maintenance (constant body weight) by underfeeding, the hypophysis usually increases slightly in gross weight; while in adult rats with acute or chronic inanition the weight of the hypophysis decreases nearly in proportion to that of the whole body. The purpose of the present paper is to present the results of an extension of the investigation, including a volumetric and histological study of the various parts of the hypophysis.

This paper forms the fourth of a series of studies upon the effects of inanition on the albino rat, the investigation being supported by a special grant from the research funds of the Graduate School of the University of Minnesota.

MATERIAL AND METHODS

The material used included the hypophyses from 91 rats, obtained partly in connection with my previous studies (Jackson '15 a and '15 b) and partly from material collected by Hoskins ('16) and Stewart ('16). The 91 rats included 44 normal (control) rats of both sexes, varying from newborn to about one year of age; 15 rats held at maintenance (constant body weight) by underfeeding beginning at the age of three weeks; 6 older rats subjected to acute inanition and 5 to chronic inanition; and 21 young rats refed for various periods after being held at maintenance from the age of three weeks to ten weeks or more.

The diet in all cases was whole wheat (graham) bread soaked in whole milk, the amount being reduced for maintenance and chronic inanition, and cut off entirely in acute inanition. Water was supplied *ad libitum* in all cases. The loss in body weight during acute and chronic inanition (adults) was about one-third.

The general data for the rats used are given in table 1. In the first column, the letter indicates the series ('H' = Hoskins; 'St.' = Stewart, etc.). The number preceding the decimal point is the litter number; the number following designates the individual rat. (This does not apply to most of the rats in subdivisions 'C' and 'D' of table 1, however, where the litters were not recorded.)

TABLE 1

General data for albino rats used

RAT NO.	SEX	AGE	NOSE-ANUS LENGTH	BODY WEIGHT GROSS (AND NET)	HYPOPHYSIS WEIGHT (FRESH)
			A. Normal rats		
		days	*mm.*	*grams*	*gram*
J 1.7a	m	Newborn		4.9	
J 1.7b	m	Newborn		4.9	
St 32.1	f	½	46	5.0	
St 72.2	m	7	66	10.8(10.0)	0.0013
St 72.5	f	7	66	10.8(10.1)	0.0012
St 80.5	f	12	75	15.5	0.0020
St 80.7	m	12	75	15.0	0.0018
J 1.2	f	21	100	28.2	0.0018
J 1.1	m	21	100	29.0	0.0020
S 7.29	f	21	107	31.5(30.4)	0.0022
S 47.1	f	21	101	33.4	0.0018
St 5.1	f	56		63.0	0.0040
St 47.6	f	67	169	123.5	0.0069
St 47.5	m	67	191	196.0	0.0065
H 70.3	m	70	194	208.1(198.3)	0.0078
H 70.7	f	71	184	162.7(155.0)	0.0099
S 11.62	f	72	170	119.3(115.2)	0.0046
H 68.11	m	72	192	170.8(163.8)	0.0068
H 68.8	f	74	184	141.6(137.0)	0.0088
H 68.3	f	74	185	158.5(151.3)	0.0084
M 1.2	m	74	190	181.1(172.6)	0.0063
S 5.4	f	74	168	125.9(117.6)	0.0050
S 5.3	m	74	180	172.0(166.5)	0.0067
H 60.3	f	78	182	138.2	0.0077
J 1.3	m	94	183	177.0	0.0063
H 65.7	f	98	179	144.1(138.9)	0.0084
H 64.3	m	101	191	184.7(179.2)	0.0071
H 59.3	f	103	196	(165.6)	0.0097
H 63.2	m	103	195	173.7(166.1)	0.0079
H 58.3	m	106	225	(258.1)	0.0089
St 12.54	f	111	166	121.0(115.9)	0.0058
St 10.24	f	112	169	134.5(129.3)	0.0099
J 1.7[1]	f	112	185	161.0	0.0092
H 50.5	f	132	196	(173.3)	0.0141
H 36.3	m	138	202	(201.6)	0.0089
H 50.3	m	141	211	(221.5)	0.0088
H 47.3	m	145	201	(167.6)	0.0060
H 34.3	f	224	194	(173.0)	0.0123
H 34.6	f	225	205	(187.5)	0.0108

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[1] At end of pregnancy.

TABLE 1—Continued

RAT NO.	SEX	AGE	NOSE-ANUS LENGTH	BODY WEIGHT GROSS (AND NET)	HYPOPHYSIS WEIGHT (FRESH)
		days	*mm.*	*grams*	*grams*
H 24.3	f	232	188	(138.4)	0.0077
H 27.3	f	253	195	(166.4)	0.0121
St 9.48	f	338	195	205.0(197.8)	0.0196
St 33.116	f	340	195	195.5(188.3)	0.0108
St 8.36	m	351	228	381.0(369.3)	0.0088
B. Rats held at maintenance from age of three weeks					
St 47.3	m	66	120	34.0	0.0022
St 47.4	m	66	113	32.3	0.0020
S 12.69	f	66	100	24.5(22.7)	0.0020
S 12.71	m	67	95	23.3(21.2)	0.0020
S 7.31	m	70	120	34.8(31.4)	0.0018
S 9.36	m	72	113	30.5	0.0018
S 6.23	f	72	102	26.1(24.5)	0.0033
S 11.63	f	72	95	23.8(22.6)	0.0022
S 5.8	f	73	105	27.6(24.6)	0.0011(?)
S 5.11	f	73	105	25.3(23.3)	0.0018
S 11.65	m	73	100	23.8(22.5)	0.0022
S 5.12	f	74	103	26.1(25.5)	0.0017
St 12.50	m	82	123	45.0(41.2)	0.0023
St 33.1	f	104	89	19.1(18.2)	0.0019
St 38.8	m	139	118	30.0	0.0020
C. Rats subjected to acute inanition					
J 1.4	m	94	180	107.0	0.0056
M 1	m	?	205	168.0(165.2)	0.0070
M 2	m	?	?	170.0(167.2)	0.0066
S 26	m	?	205	174.0(171.5)	0.0076
S 16	f	?	195	190.0(186.0)	0.0146
S 27	m	?	215	223.0(219.0)	0.0096
D. Rats subjected to chronic inanition					
J 1.5	m	117	175	97.0	0.0042
M 12	m	?	173	128.0(124.6)	0.0052
M 5	m	?	190	129.0(126.3)	0.0058
M 6	m	?	175	138.0(134.1)	0.0060
M 11	m	?	190	163.0(158.5)	0.0064
E 1. Rats refed one-half week after maintenance (three to twelve weeks)					
St 12.53	m	85	123	48.5(44.9)	0.0028
St 10.25	f	87	113	40.5(36.5)	0.0022

TABLE 1—Concluded

RAT NO.	SEX	AGE	NOSE-ANUS LENGTH	BODY WEIGHT GROSS (AND NET)	HYPOPHYSIS WEIGHT (FRESH)
E 2. Rats refed one week after maintenance (three to twelve weeks)					
		days	*mm.*	*grams*	*grams*
St 11.41	m	88	132	62.2(58.2)	0.0031
St 11.42	f	88	125	55.0(50.3)	0.0031
St 11.45	f	88	130	67.6(57.7)	0.0034
St 12.48	f	88	125	50.2(46.5)	0.0028
St 10.27	f	89	127	55.0(51.4)	0.0028
E 3. Rats refed two weeks after maintenance (three to twelve weeks)					
St 11.40	f	95	142	84.5(76.5)	0.0030
St 11.43	f	95	143	79.0(74.7)	0.0034
St 12.49	f	95	129	60.5(55.5)	0.0027
St 12.51	f	95	137	77.2(70.7)	0.0034
St 10.26	m	96	150	91.0(86.3)	0.0033
E 4. Rats refed four weeks after maintenance (three to twelve weeks)					
St 12.52	m	109	171	125.2(117.4)	0.0056
St 12.47	f	109	161	106.5(98.0)	0.0082
St 11.44	f	109	151	94.2(88.4)	0.0058
St 11.46	f	109	163	118.0(110.1)	0.0088
St 10.28	f	110	167	118.0(109.5)	0.0068
St 10.23	f	111	168	129.5(119.1)	0.0094
E 5. Rat refed one year after maintenance (three to ten weeks)					
S 14.3	m	444	236	332.0(318.9)	0.0097
E 6. Rats refed after maintenance from age of three to twenty weeks					
S 33.120	f	339	181	162.0(155.9)	0.0096
S 33.118	m	346	204	229.0(218.5)	0.0094

The material was obtained immediately after the animals were killed (by chloroform). Especial care must be taken to avoid injury in the removal of delicate organs like the hypophysis. The glands were in nearly all cases fixed in Zenker's fluid and stained with haematoxylin-eosin. Formalin and Zenker-formol were tried as fixatives, and iron-haematoxylin as a stain, with less satisfactory results. The glands were embedded in paraffin, and cut in sections (usually serial) 3 μ to 5 μ in thickness. The sections were cut in the frontal (coronal) plane.

The volumes of the various parts (lobes) of the hypophysis were determined by a method similar to that used by Hammar ('14) for the thymus. The outlines of the sections, magnified 75 diameters, were projected upon "American Linen Record" paper (sheets 18 x 23 inches, 36 lbs. per ream) by means of an Edinger projection apparatus. Four samples, each 5 cm. square, were weighed from each sheet, and the area corresponding to each gram of paper determined. The various lobes as outlined were then cut out and weighed, and the corresponding areas calculated. This magnified area was then reduced to actual area and multiplied by the thickness of the sections, giving the actual volume for each lobe. By trial it was found to be unnecessary to draw every section, about 50 sections taken at equal intervals (every fifth to tenth section) being found to give nearly identical results.

On comparing the original weight of the hypophysis with the volume obtained by the preceding method, there is apparently a marked discrepancy, the volume (in cubic centimeters) being less than half the weight (in grams) (table 2). The difference is due: (1) to the density of the gland; (2) to the capsule and extracapsular structures attached, which were weighed but not included in the volume measured; (3) to the great shrinkage resulting from the process of fixation, dehydration and embedding in paraffin. This process alone would probably account for a shrinkage of nearly one-third in volume.

For the pars anterior (distalis), a plan similar to that above given for the lobes was followed to determine the relative volumes of the vascular stroma and the parenchyma; and of the nuclei and cytoplasm in the parenchyma. For this purpose, a higher magnification (Zeiss 2 mm. apochromat with compensating ocular 6, giving magnification of 1420 diameters at table level) was used. A typical field was chosen, and as large an area as possible drawn, with the aid of a Spencer camera lucida. It is important that the section drawn represent as nearly as possible a true optical plane, and therefore no change of focus during the drawing is permissible. If this is not carefully done, there will be a tendency to draw the nuclei too large, since their maximum diameter will frequently lie outside the optical plane chosen. On account of the unavoidable spherical aberration, a larger field will be available in a single optical plane if the section is a little thicker, hence 5 μ is preferable to 3 μ for this purpose.

Four different fields from typical regions were thus drawn in each case, and the average results taken (table 3). The total number of nuclei in the four fields varied from 269 (in St 47.5) to 509 (in S 6.23). If we assume that the nuclei are spherical (which is, of course, not strictly true), the average area per nucleus in a given optical plane should, according to the rules of solid geometry, represent two-thirds the area of the corresponding great circle. (The volume of a sphere equals two-thirds of the volume of the circumscribed cylinder.) Upon this assumption, the average nuclear diameter was calculated (table 3). The corresponding average cell diameter was also calculated, assuming that the cytoplasm surrounds the nucleus in a layer of uniform thickness.

The diameters of nuclei and cells thus calculated can of course be considered only as roughly approximate. So far as the nuclei are concerned, however, the results were controlled by a series of direct measurements (with a filar wheel-micrometer eyepiece). In this case, the measurements were not restricted to a single optical plane, but the maximum nuclear diameters were obtained by focussing. The average nuclear diameters thus measured are given in table 4. Some variability is naturally to be expected, but the results by the two independent methods are seen to be in fair agreement.

RELATIVE VOLUMES OF THE PARTS (LOBES)

a. Normal growth

It will be noted in table 2 that the 'volume of lobes' is usually slightly less than the corresponding 'volume of gland.' The difference represents the volume of the hypophyseal cavity. This is relatively small (fig. 1), rarely exceeding 3 per cent of the total volume, and is not appreciably affected by inanition. Since the hypophysis was removed by division of the infundibulum, it is evident that the portions described by Tilney ('13) in the floor of the third ventricle (eminentia saccularis and pars tuberalis) are not included in the parts examined.

From table 2 A it is evident that during normal postnatal growth there is considerable individual variation in the relative size of the various lobes. On comparing the younger (newborn to three weeks) with the older rats (ten weeks and above), however, it appears that in general the pars anterior becomes relatively larger, the pars nervosa correspondingly smaller. The pars intermedia is variable, but in general apparently undergoes no definite change in relative size in either direction.

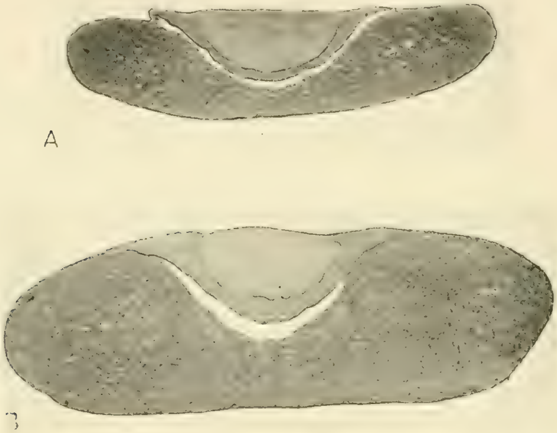


Fig. 1 From photographs representing frontal (coronal) sections through the normal hypophysis of the albino rat (A) at three weeks (rat J-1.2) and (B) at ten weeks (St 47.5). The hypophyseal cavity (residual lumen) and the three parts (anterior, intermedia and nervosa) are apparent. The anterior lobe is relatively larger in the older rat. × 25.

That the hypophysis is relatively heavier in the female rat is already known (Hatai '13). The present data indicate that this is due chiefly, if not entirely, to a larger anterior lobe in the female. Comparing the 4 older males in table 2 A with the 4 corresponding females, we find the following averages:

	NOSE-ANUS LENGTH	BODY WEIGHT	HYPOPHYSIS WEIGHT	PARS ANT.	PARS INT.	PARS NERV.
	mm.	*grams*	*grams*	*per cent*	*per cent*	*per cent*
Males	190	188	0.00685	82.0	9.7	8.3
Females	179	148	0.00808	86.4	6.7	7.0

Thus in the females the pars anterior appears to have gained, while the pars intermedia and, to a lesser extent, the pars nervosa, have decreased in relative (percentage) weight.

The question naturally arises as to whether the increased relative size of the anterior lobe in the female is sufficient alone to account for the known sexual difference in the weight of the gland. In the averages above shown, the higher percentage weight in the hypophysis of the female is more than sufficient to account for the sexual difference in the gross weight of the gland upon this basis. But this disregards the fact that in these cases the body weight is higher in the males, which might alter the relations. If, however, we compare female H 70.7 and male H 68.11, whose ages and body weights are not very different (see table 1), we still find that the larger hypophysis of the female (0.0099 gram) as compared with that of the male (0.0068 gram) may be accounted for as due chiefly to the heavier anterior lobe in the former (86.2 per cent by volume, as against 80.7 per cent in the male), as shown in table 2. Or, comparing the absolute volumes (not given in the table), in the hypophysis of the male H 68.11 the volumes of the partes anterior, intermedia and nervosa were 0.00241 cc., 0.00033 cc. and 0.00025 cc., respectively; while in female H 70.7 the corresponding volumes were 0.00386 cc., 0.00034 cc. and 0.00029 cc. Thus the larger hypophysis of the female showed but slight increase in the partes intermedia and nervosa, but a very large increase in the pars anterior.

It should further be noted, however, that in the rats used the sexual difference in the weight of the hypophysis is not so great as that shown in Donaldson's ('15) tables for rats of corresponding weight or body length. To produce this difference would require a greater preponderance of relative size for the anterior lobe of the female than is found in my measurements. It is

TABLE 2

Volumetric data on the parts (Lobes) of the hypophysis of the albino rat under various conditions

RAT NO.	SEX	AGE	GROSS BODY WEIGHT	HYPOPHYSIS WEIGHT	VOLUME OF GLAND	VOLUME OF LOBES	PERCENTAGE FORMED BY		
							Pars anterior	Pars intermedia	Pars nervosa
A. Normal rats (controls)									
		days	*grams*	*grams*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*
J 1.7a	m	Nb	4.9		0.0001745	0.0001599	77.7	10.5	11.5
J 1.1	m	21	29.0	0.0020	0.0006494	0.0006480	76.9	8.0	15.0
St 47.5	m	67	196.0	0.0065	0.003172	0.003085	85.3	6.0	8.7
H 70.3	m	70	208.1	0.0078	0.003662	0.003662	79.8	11.9	8.3
H 68.11	m	72	170.8	0.0068	0.003068	0.002993	80.7	11.0	8.3
J 1.3	m	94	177.0	0.0063	0.002844	0.002818	82.3	9.7	8.0
J 1.2	f	21	28.2	0.0018	0.0007877	0.0007597	79.2	8.0	12.0
S 47.1	f	21	33.4	0.0018	0.0008064	0.0007913	81.0	6.0	13.0
St 47.6	f	67	123.5	0.0055	0.002946	0.002868	86.9	6.0	7.4
H 70.7	f	71	162.7	0.0099	0.004598	0.004485	86.2	7.5	6.5
J 1.7*	f	112	161.0	0.0092	0.004346	0.004300	87.0	5.7	7.3
H 24.3	f	232	145.0	0.0077	0.003685	0.003568	85.4	7.7	6.9
B. Rats held at maintenance from age of three weeks									
St 47.4	m	66	32.3	0.0020	0.0008340	0.0008199	76.1	7.9	15.9
St 47.3	m	66	34.0	0.0022	0.0008609	0.0008417	75.2	9.0	16.0
St 12.50	m	82	45.0	0.0023	0.001053	0.001053	71.0	10.5	18.5
C. Adult rats with acute inanition									
S 27	m		223.0	0.0096	0.003957	0.003900	84.4	6.5	9.0
S 16	f		190.0	0.0078	0.005723	0.005665	89.6	3.5	6.9
D. Adult rats with chronic inanition									
M 12	m		128.0	0.0052	0.002491	0.002467	79.0	7.0	14.0
E 1. Rats refed one-half week after maintenance from three to twelve weeks of age									
St 10.25	f	87	40.5	0.0022	0.001080	0.001060	75.6	7.0	17.5
E 2. Rats refed one week after maintenance from three to twelve weeks of age									
St 11.41	m	87	62.2	0.0031	0.001558	0.001462	78.3	8.0	13.7
St 10.27	f	89	55.0	0.0028	0.001534	0.001494	81.0	7.0	12.0
St 11.45	f	88	67.6	0.0034	0.001431	0.001405	82.0	7.0	11.0

* Rat No. J 1.7 had just given birth to a (first) litter.

TABLE 2—Concluded

RAT NO.	SEX	AGE	GROSS BODY WEIGHT	HYPOPHYSIS WEIGHT	VOLUME OF GLAND	VOLUME OF LOBES	PERCENTAGE FORMED BY		
							Pars anterior	Pars intermedia	Pars nervosa
E 3. Rats refed two weeks after maintenance from three to twelve weeks of age									
		days	*grams*	*gram*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*
St 10.26	m	96	91.0	0.0033	0.001843	0.001803	78.6	7.0	14.2
St 11.40	f	95	84.5	0.0030	0.001496	0.001443	81.6	6.0	12.4
St 11.43	f	95	79.0	0.0034	0.001680	0.001643	79.0	7.0	14.0
St 12.51	f	95	77.2	0.0034	0.001362	0.001347	79.2	6.8	14.0
E 4. Rats refed four weeks after maintenance from three to twelve weeks of age									
St 12.47	f	109	106.5	0.0082	0.002608	0.002608	86.7	4.5	8.8
E 5. Rats refed six or seven months after maintenance to age of about five months									
S 33.118	m	346	229.0	0.0094	0.004155	0.004086	78.0	6.7	15.2
S 33.120	f	339	162.0	0.0096	0.003981	0.003927	78.1	7.0	14.0

therefore possible that further data would modify my provisional conclusion that the larger gland in the female is due to a larger anterior lobe.

The pars intermedia averages somewhat higher in the males than might be expected according to the theory above mentioned (that the sexual difference in hypophysis weight is due to difference in the anterior lobe alone), but this is probably due to the unusual individual variations, which table 2 shows to occur in the pars intermedia.

It may be noted that in the one rat (J 1.7) killed at the end of pregnancy, the expected hypertrophy of the gland apparently failed to occur, and no marked change is evident in the relative size of the three parts (lobes). The other females used were all virgins.

b. Volumes of the lobes in young rats held at maintenance

As shown in table 2 B, in the 3 young rats held at maintenance (nearly constant body weight) from three to ten or twelve weeks, the pars anterior has been reduced in relative volume, the partes intermedia and nervosa becoming correspondingly larger. This change is especially well marked in the rat held at maintenance up to twelve weeks of age.

c. Volumes of the lobes in adult rats subjected to acute and chronic inanition

The 2 rats, S 27 and S 16, had been subjected to acute inanition (water but no food given) for about ten days, with resultant loss of about 30 per cent in body weight. The final body weights are given in table 1. The pars anterior of the hypophysis in these rats appears relatively somewhat larger than normal, the pars intermedia somewhat smaller, the pars nervosa not much changed in relative size (table 2 C).

In the rat (M 12) subjected to chronic inanition (progressive underfeeding for thirty-six days, with loss of 36 per cent in body weight), the partes anterior and intermedia are smaller and the pars nervosa larger than normal in relative size (table 2 D). Thus the relative changes in the size of the lobes during chronic inanition of the adult appear in general to resemble more nearly the changes in the young during maintenance, which are somewhat different from those during acute inanition. The general resistance of the pars nervosa during inanition recalls the similar behavior of the closely related brain.

d. Volumes of the lobes in rats refed after maintenance

As seen in table 2 (E 1, E 2, E 3 and E 4), in the young rats refed one-half week, one week, two weeks and four weeks, there is a considerable individual variation in the relative size of the parts (lobes) of the hypophysis. In general, however, there appears a gradual return to the normal proportions, the pars anterior increasing and the partes intermedia and nervosa decreasing in relative (percentage) volume. The exceptionally small pars intermedia in the rat refed 4 weeks is probably an individual variation.

Two rats (table 2 E 5) were refed six or seven months after a long period of maintenance, from the age of three weeks to five months. In these cases, although the body weights and hypophysis weights are nearly normal, the lobes are abnormal in their relative size. In each the pars anterior is relatively small, and the pars nervosa large. This resembles the change found after maintenance in young rats and chronic inanition in older rats. It is therefore probably a persistent effect of the inanition during the prolonged period of maintenance.

VOLUMETRIC ANALYSIS OF TISSUES AND CELLS IN PARS ANTERIOR

As shown in table 3, data were obtained in eight cases by the method previously described. A larger number of observations would doubtless reveal much individual variation, so that no great emphasis can be laid upon the exact figures in the data obtained. These quantitative data confirm the general impressions noted in the much larger number of cases studied, however, so that the general conclusions may be regarded as fairly certain.

TABLE 3

Volumetric data on the component parts (tissues and cells) in the pars anterior (distalis) of the hypophysis of the albino rat under various conditions.

RAT NO.	SEX	AGE AND CONDITION	VESSELS AND STROMA	PARENCHYMA	PERCENTAGE OF PARENCHYMA FORMED BY		PARENCHYMA CELLS AVERAGE DIAMETER (CALCULATED)	
					Nuclei	Cytoplasm	Nuclei μ	Cell μ
			per cent	*per cent*	*per cent*	*per cent*		
J 1.7a	m	Normal newborn	6.7	93.3	34.1	65.9	5.9	10.1
St 47.1	f	Normal 21 days	9.6	90.4	24.1	75.9	5.8	11.9
St 47.5	m	Normal 67 days	10.6	89.4	19.7	80.3	6.0	13.6
S 5.12	f	Maintained 3–10 weeks	13.4	86.6	25.9	74.1	5.0	9.7
S 6.23	f	Maintained 3–10 weeks	8.8	91.2	23.4	76.6	4.9	10.2
St 33.1	f	Maintained 3–15 weeks	13.4	86.6	28.4	71.6	5.3	10.0
S 27	m	Adult acute inanition	16.7	83.3	25.7	74.3	5.5	10.8
M 12	m	Adult chronic inanition	17.5	82.5	23.1	76.9	5.3	11.0

a. Relative volumes of stroma and parenchyma (table 3)

In the pars anterior of the normal newborn rat, the vessels and associated stroma form 6.7 per cent of the total volume, increasing to 9.6 per cent at three weeks and to 10.6 per cent at ten weeks (sixty-seven days). At this time the gland appears to have reached its maximum normal vascularity; though no actual measurements were made upon later stages, and there is naturally some individual variation.

In young animals held at maintenance, there is usually a striking increase in the vascularity, the sections showing a marked hyperemic appearance. This is confirmed by the measurements on two of the three cases in table 3, both showing an increase of the vascular stroma to 13.4 per cent. The third case represents an unusual condition in which, on the other hand, there appears to be a slight decrease to 8.8 per cent in the volume of the vessels and associated stroma.

In adult rats subjected to acute or chronic inanition the hyperemia is usually even more conspicuous than in the younger animals. This is confirmed by the measurements, showing for the vascular stroma 16.7 per cent by volume for the case of acute inanition and 17.5 per cent for the chronic inanition. These may be considered as typical, though here also individual variations occur.

In general, therefore, it appears that during inanition in the pars anterior the parenchyma becomes reduced in relative volume, with corresponding increase in the vascular stroma. This increase is due chiefly to a distention of the blood-vessels, giving the sections a markedly hyperemic appearance. In a few cases, especially in the young held at maintenance, there is also some increase in the intercellular substance.

b. Relative volumes of the nuclei and cytoplasm of the parenchyma (table 3)

In the parenchyma of the pars anterior, the nuclei form 34.1 per cent of the total cell volume in the newborn, decreasing to 24.1 per cent at three weeks and to 19.7 per cent at ten weeks (sixty-seven days). The cytoplasm, of course, undergoes a corresponding increase in relative volume (figs. 2 and 4). The relations found in the specimen at ten weeks (St 47.5) appear to be typical for adults, although no actual measurements were made at later stages. Some allowance must also be made for variations, both in different individuals and in different parts of the gland in the same individual.

In 2 of the 3 young rats held at maintenance, in which the volumetric data were obtained, there appears to be a relative increase in the nuclear volume to 25.9 per cent and 28.4 per cent respectively. This involves a corresponding loss in relative volume for the cytoplasm, which is doubtless typical (fig. 3). Individual variations occur, however, as in the third case (S 6.23, which also had a small stroma volume), in which the nuclei formed 23.4 per cent by volume, or slightly less than the normal at three weeks.

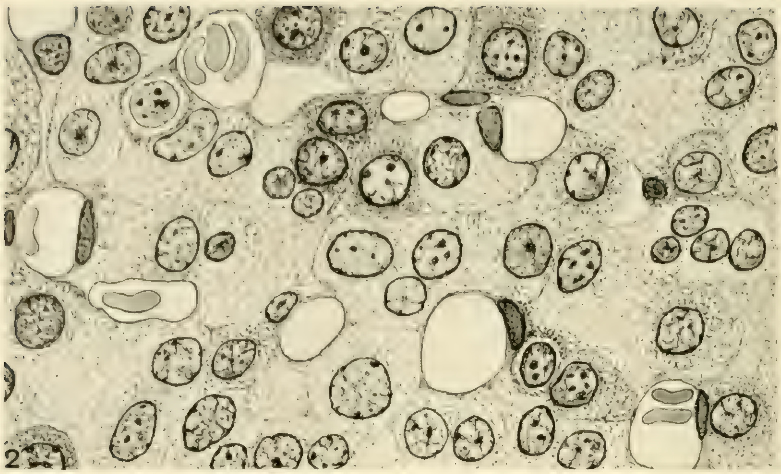
In the two older rats subjected to chronic and acute inanition the relative increase in nuclear volume (with corresponding cytoplasm decrease) appears even more strongly marked, reaching 23.1 and 25.7 per cent respectively, in comparison with 19.7 per cent for the normal. The crowding of the nuclei with reduction of cytoplasm is evident in figure 5. Allowance must of course be made for variations, but the general tendency is unmistakable.

c. *Estimated size of cells and nuclei*

The average diameters of the cells and nuclei of the parenchyma in the pars anterior, calculated as explained under "Material and Methods," are given in the last two columns of table 3. The average cell diameters by this method appear to increase from 10.1 μ in the newborn to 11.9 μ at three weeks and 13.6 μ at ten weeks (sixty-seven days). This appears to represent the average permanent adult size, although individual variations occur.

In the young rats held at maintenance, the average cell diameters in the three cases measured by this method range from 9.7 μ 10.2 μ, or considerably less than the normal at three weeks (11.9 μ). In the adults subjected to acute and chronic inanition, the cell diameters are 10.8 μ and 11.0 μ, likewise a marked reduction in comparison with the normal (13.6 μ).

The corresponding average nuclear diameters, estimated by this method, show very little change with age in the normal rats,



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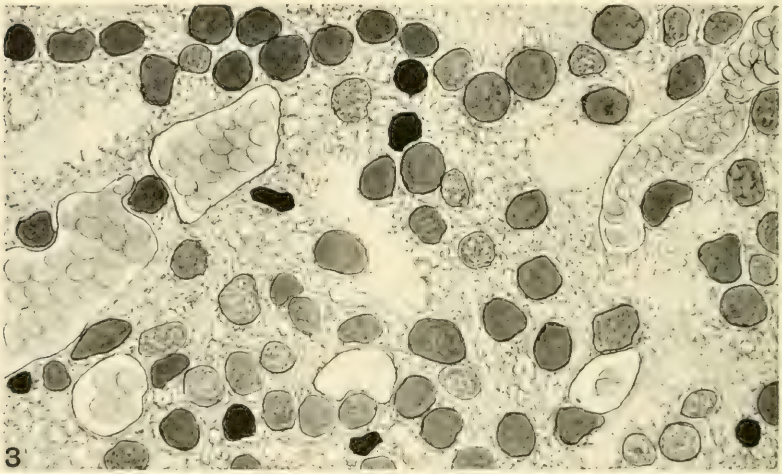

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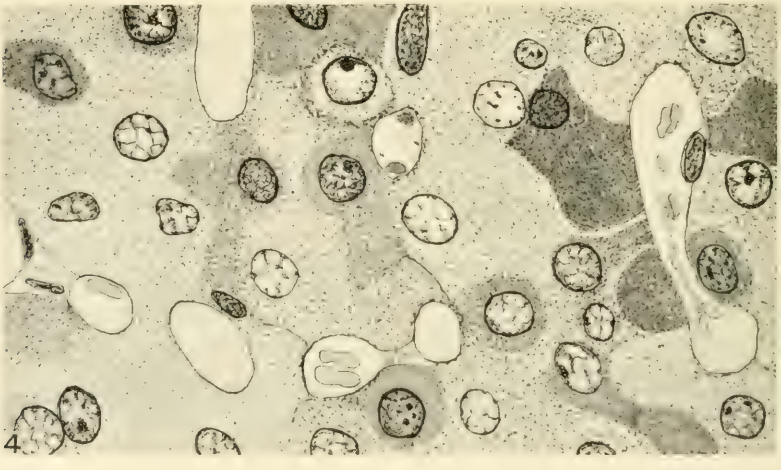

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being 5.9 μ in the newborn, 5.8 μ at three weeks and 6.0 μ at ten weeks (sixty-seven days). In the three young rats held at maintenance, the nuclear diameter has diminished, varying from 4.9 μ to 5.3 μ. In the 2 older starved rats the corresponding diameters are 5.3–5.5 μ.

DIRECT MEASUREMENT OF NUCLEAR DIAMETERS

Owing to the imperfections of the volumetric method previously considered, and to the small number of cases to which it was applied, the results should be considered as only approximate and not final. So far as the nuclear diameters are concerned, however, the results have been controlled by a totally different method, that of direct measurement of the nuclear diameters with a filar micrometer eyepiece. Where the nuclear diameters are unequal (slightly ellipsoidal forms are frequent), the average of the longer and shorter diameters was taken. Nuclei apparently cut near the edge, so as to leave the maximum extent outside the section, were avoided so far as possible. The average (and range) for 100 nuclei in each case is shown in table 4.

The results of these direct nuclear measurements are more uniform, and in closer agreement with those of the volumetric method, than might be expected. This will appear upon a com-

Fig. 2 A small portion of the pars anterior of the hypophysis in a normal rat at three weeks (J 1.2). Most of the cells are of the faintly basophilic type. Some eosinophilic cells are indicated by darker staining (two near the upper margin of the figure). A few chromophobic cells are shown, a group of four near the right margin. Zenker fixation; haematoxylin stain. Drawn with the aid of a camera lucida. × 950.

Fig. 3 A small portion of the pars anterior of the hypophysis in a young rat (S 5.12) held at maintenance from age of three to ten weeks. The inanition effect is very marked, with hyperemia and atrophy of the parenchyma. Cytoplasm diminished in amount, sparsely granular and filled with coarse vacuoles which in places coalesce to form irregular spaces. Nuclei hyperchromatic, in various stages of pycnosis. Zenker fixation; haematoxylin-eosin stain. Drawn with the aid of a camera lucida. × 950.

Fig. 4 A small portion of the pars anterior of the hypophysis in a normal rat aged ten weeks (St 47.5). This represents the typical adult structure. Most of the cells are of the weakly basophilic type. Several eosinophiles are indicated, the more strongly acidophilic having a darker color. Zenker fixation; haematoxylin-eosin stain. Drawn with the aid of a camera lucida. × 950.

parison of tables 3 and 4. The direct nuclear measurements were made in a larger number of cases (12) and therefore give a somewhat better idea of the amount of individual variation.

The direct measurements of nuclei, as shown in table 4, were made for the pars intermedia as well as the pars anterior. Considering the latter first, measurements were in most cases made separately for the eosinophile and non-eosinophile (including basophile and chromophobe) cells. In the earlier normal stages there does not appear to be any constant difference in nuclear size between the two classes of cells. But later, and especially in the rats subjected to inanition, the average is less for the nuclei of eosinophiles. This is probably because the eosinophiles appear more prone to karyopycnosis.

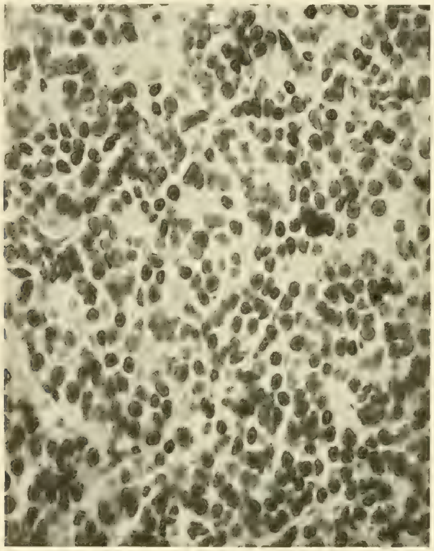

Fig. 5. From a photograph showing a small part of the anterior lobe of the hypophysis in an adult rat (M 12) subjected to chronic inanition. Marked effects of inanition: hyperemia; atrophy and loss of specific staining reactions in cytoplasm; hyperchromatism and pycnosis of nuclei. Zenker fixation; haematoxylin-eosin stain. × 440.

The nuclei of the pars intermedia cells do not differ much in size from those of the pars anterior, especially of the non-eosino-

TABLE 4

Average diameter of nuclei of parenchyma cells in the hypophysis of the albino rat under various conditions. Direct measurements by means of the filar micrometer. Average (and range) of 100 nuclei in each case

RAT NO.	SEX	AGE AND CONDITION	PARS ANTERIOR (DISTALIS)		PARS INTERMEDIA
			Non-eosinophiles	Eosinophiles	
			μ	μ	μ
J 1.7a	m	Normal newborn	[1]5.8(3.4–7.3)		5.8(3.4–7.5)
J 1.1	m	Normal 21 days	5.7(4.2–6.9)	5.8(4.5–6.8)	5.7(5.0–6.8)
St 47.1	f	Normal 21 days	5.6(4.0–7.0)	5.7(4.5–6.6)	5.4(4.0–7.0)
St 47.5	m	Normal 67 days	5.7(4.5–6.6)	5.7(4.8–6.3)	5.8(5.1–7.1)
St 47.6	f	Normal 67 days.	6.0(4.0–7.5)	5.7(3.8–6.7)	5.8(4.8–7.1)
H 34.6	f	Normal 225 days	5.9(4.7–6.7)	5.4(4.3–6.3)	6.1(5.0–7.2)
S 6.23	f	Maintained 3–10 weeks	5.6(4.2–6.9)	5.2(3.5–6.1)	5.4(4.4–7.2)
S 5.12	f	Maintained 3–10 weeks	[1]5.4(4.1–6.5)		5.4(4.4–6.2)
St 33.1	f	Maintained 3–15 weeks	[1]5.3(3.7–6.9)		5.8(4.5–6.6)
S 27	m	Adult acute inanition	5.3(4.0–6.4)	5.1(3.6–6.4)	5.5(4.5–6.6)
M 1	m	Adult acute inanition	5.2(3.5–6.2)	5.3(3.5–6.4)	5.7(4.3–6.9)
M 12	m	Adult chronic inanition	5.2(3.7–6.5)	4.7(3.1–5.7)	5.3(3.3–6.1)

[1] Including some eosinophiles.

philes. During inanition they show in most cases less shrinkage than do the pars anterior nuclei (especially of eosinophiles), though here as elsewhere much individual variation is found.

OCCURRENCE OF MITOSIS IN THE PARTS (LOBES)

In order to determine the rate of mitosis in the hypophysis of the rat under various conditions, the number of mitoses was counted in 40 cases, as shown in table 5. In each case, one or more (up to 5) entire coronal (frontal) sections, chosen to show maximum areas (especially of partes intermedia and anterior) were systematically examined by means of a mechanical stage, and the total number of mitoses counted for each part (lobe). As might be expected, much individual variation is found, both in different individuals and in different sections of the same gland. The general trend, however, is clear and is well shown in the condensed table of averages (table 6). Amitosis was not observed in any case.

TABLE 5

Mitosis in the hypophysis of the albino rat under various conditions

RAT NO.	SEX	AGE	GROSS BODY WEIGHT	HYPOPHYSIS WEIGHT	NUMBER OF MITOSES COUNTED IN ENTIRE CORONAL SECTIONS OF THE HYPOPHYSIS IN THE		
					Pars anterior	Pars intermedia	Pars nervosa
A. Normal rats							
		days	*grams*	*grams*			
J 1.7a	m	nb.	4.9		46; 68; 67; 66	8; 8; 13; 5	14; 4; 5; 6
St 72.5	f	7	10.8	0.0012	16; 15; 18; 23; 20	0; 0; 1; 1; 2	1; 1; 0; 0; 0
St 80.5	f	12	15.5	0.0020	16	0	0
J 1.1	m	21	29.0	0.0020	5; 7; 7; 11; 7	2; 3; 1; 2; 3	0; 0; 0; 0; 0
S 7.29	f	21	31.5	0.0022	7; 10; 8; 9; 5	1; 1; 1; 2; 2	0; 0; 0; 0; 0
S 47.1	f	21	33.4	0.0018	5; 6; 6; 6; 6	1; 0; 1; 0; 2	0; 0; 0; 0; 0
St 47.6	f	67	123.5	0.0055	2; 4; 5; 7; 2	0; 0; 0; 0; 0	0; 0; 0; 0; 0
St 47.5	m	67	196.0	0.0065	0; 0; 0	0; 0; 0	0; 0; 0
H 68.11	m	72	170.8	0.0068	2	0	0
S 5.3	m	74	172.0	0.0067	3; 2; 1; 1	0; 1; 0; 0	0; 0; 0; 0
J 1.3	m	94	177.0	0.0063	10; 12; 8	1; 1; 2	0; 0; 0
H 63.2	m	103	173.7	0.0079	0; 2	0; 1	0; 0
St 12.54	f	111	121.0	0.0058	0; 2	0; 0	0; 0
H 34.6	f	225	195.0	0.0108	0; 0; 1	0; 0; 0	0; 0; 0
B. Rats held at maintenance from age of three weeks							
St 47.3	m	66	34.0	0.0022	0; 0; 0; 1 ; 0;	0; 0; 0; 0; 0	0; 0; 0; 0; 0
St 47.4	m	66	32.3	0.0020	0; 0; 0; 1 ; 0	0; 0; 0; 0; 0	0; 0; 0; 0; 0
S 6.23	f	72	26.1	0.0033	0	0	0
S 5.11	f	73	25.3	0.0018	0	0	0
S 11.65	m	73	23.8	0.0022	0	0	0
S 5.12	f	74	26.1	0.0017	0	0	0
St 33.1	f	104	19.1	0.0019	1; 1; 0; 0; 0	0; 0; 0; 0; 0	0; 0; 0; 0; 0
St 38.8	m	139	30.0	0.0020	1; 1; 0; 0; 0	0; 0; 0; 0; 0	0; 0; 0; 0; 0
C 1. Rats refed one-half week after maintenance from three to twelve weeks of age							
St 12.53	m	85	48.5	0.0028	2; 2	0; 0	0; 0
St 10.25	f	87	40.5	0.0022	1; 2	0; 0	0; 0
C 2. Rats refed one week after maintenance from three to twelve weeks of age							
St 11.41	m	88	62.2	0.0031	12; 7; 10	0; 1; 0	0; 0; 0
St 10.27	f	89	55.0	0.0028	6	0	0
St 12.48	f	88	50.2	0.0028	7	0	0
St 11.45	f	88	67.6	0.0034	6	0	0
St 11.42	f	88	55.0	0.0031	2; 2	0; 0	0; 0

TABLE 5—Concluded

RAT NO.	SEX	AGE	GROSS BODY WEIGHT	HYPOPHYSIS WEIGHT	NUMBER OF MITOSES COUNTED IN ENTIRE CORONAL SECTIONS OF THE HYPOPHYSIS IN THE		
					Pars anterior	Pars intermedia	Pars nervosa
C 3. Rats refed two weeks after maintenance from three to twelve weeks of age							
		days	*grams*	*grams*			
St 12.51	f	95	77.2	0.0034	8	0	0
St 12.49	f	95	60.5	0.0027	5	0	0
St 11.40	f	95	84.5	0.0030	13; 8	0; 0	0; 0
St 11.43	f	95	79.0	0.0034	6	0	0
St 10.26	m	96	91.0	0.0033	5	0	0
C 4. Rats refed four weeks after maintenance from three to twelve weeks of age							
St 10.28	f	110	118.0	0.0068	1; 1	0; 1	0; 0
St 10.23	f	111	129.5	0.0094	5	0	0
St 12.47	f	109	106.5	0.0082	3	0	0
St 12.52	m	109	125.2	0.0056	5	0	0
St 11.46	f	109	118.0	0.0088	107; 112	0; 0	0; 0
C 5. Rat refed six months after maintenance from three to twenty weeks of age							
S 33.118	m	346	229.0	0.0094	0	0	0

a. During normal growth of the hypophysis

To determine the rate of mitosis in the normal gland at various ages, sections were examined from 14 individuals, varying from newborn to 225 days of age (tables 5 and 6). In the newborn, mitosis is very active throughout the gland, the average being 62 mitoses in each section for the pars anterior, 9 for the pars intermedia, and 7 for the pars nervosa. Considering the difference in the areas of the three lobes (fig. 1), it may be concluded that the actual number of mitoses for a given number of cells (the 'mitotic index' of Minot) would probably be similar in all three lobes, though somewhat less in the pars nervosa.

In the pars nervosa, mitoses soon disappear. From an average of 7 per section in the newborn, they decrease to an average of two-fifths (i.e. 2 mitoses found in 5 sections) at seven days. In later stages (twelve days and above) none was found in any case. Growth in the pars nervosa thereafter consists almost entirely in the accumulation of intercellular substances.

In the pars intermedia mitosis continues, but at a progressively decreasing rate. The decrease is even relatively greater than is apparent, on account of the progressive increase in the absolute size of the areas examined. The average of 9 mitoses per section in the newborn decreases to a little less than 1 at one week and three weeks, and to one-thirteenth at ten weeks.

TABLE 6

Summary of number of mitoses in sections of the hypophysis. Averages from table 5 (exceptional cases J 1.3 and St 11.46 being excluded)

AVERAGE AGE	NUMBER OF EACH SEX IN GROUP	CONDITION	AVERAGE NUMBER OF MITOSES PER SECTION IN		
			Pars anterior	Pars inter-media	Pars nervosa
days					
Newborn	1 m	Normal	62	9	7
7	1 f	Normal	18	$\frac{4}{5}$	$\frac{2}{5}$
21	1 m, 2 f	Normal	7	$\frac{22}{25}$	0
70	3 m, 1 f	Normal	2	$\frac{1}{13}$	0
145	1 m, 2 f	Normal	$\frac{5}{7}$	$\frac{1}{7}$	0
83	4 m, 4 f	Maintained from 3 weeks	$\frac{1}{4}$	0	0
86	1 m, 1 f	Refed $\frac{1}{2}$ week	$1\frac{3}{4}$	0	0
88	1 m, 4 f	Refed 1 week	7	$\frac{1}{8}$	0
95	1 m, 4 f	Refed 2 weeks	7	0	0
110	1 m, 3 f	Refed 4 weeks	3	$\frac{1}{7}$	0
346	1 m	Refed 6 months	0	0	0

It apparently increases somewhat in the older cases (even excluding J 1.3, which also shows an abnormally large number in the pars anterior), bringing the average up to one-seventh. This apparent increase is probably due to chance variation in the relatively small number of sections examined.

In the pars anterior, the rate of mitosis likewise decreases (tables 5 and 6), the average number being 62 at birth, 18 at one week, 7 at three weeks, 2 at ten weeks and five-sevenths in 3 older rats. From the last group is excluded one very exceptional case (J 1.3, 94 days) in which the number of mitoses varied from 8 to 12 per section. This represents an extreme individual variation. At the other extreme is St 47.5 (at 67 days) in which no mitoses were found in 3 entire sections. In this case the body weight was unusually high (196 grams), however, and growth had probably become very slow as the adult weight was approached. It is probable that occasional mitoses occur so long as growth continues.

b. Mitoses in young rats held at maintenance, and in adults with acute and chronic inanition

In the young rats held at maintenance from the age of three weeks to ten weeks or more, mitosis has nearly ceased (tables 5 and 6). No mitoses were observed in the partes intermedia and nervosa. In the pars anterior, however, mitoses still occur occasionally, the total number found in 24 sections being 6, or an average of one-fourth per section. It is of interest to note that occasional mitosis in the pars anterior persisted even in a rat held nearly at maintenance from three weeks to twenty weeks of age (St 38.8), and which was nearly dead from inanition when killed. In spite of this persistent mitosis, however, the pars anterior apparently suffers the greatest relative loss in volume, as previously shown.

No mitoses were observed in any of the older rats subjected to acute and chronic inanition. No systematic search was made for them over entire sections, however, so they are not included in the tables.

c. Mitoses in various stages of refeeding after maintenance

In the young rats refed after a period of maintenace (tables 5 C and 6), mitoses were never found in the pars nervosa. In the pars intermedia, in rats refed one to four weeks, they occur occasionally, about as often as in younger normal rats of corresponding weight. In the pars anterior, mitosis reappears promptly. In rats refed only one-half week, in 4 sections examined a total of 7 mitoses was observed (table 5 C 1), or an average of one and three-fourths per section. The rate increases to an average of about 7 mitoses per section in rats refed one week and two weeks, and about 3 in those refed four weeks.

No mitoses were found in one rat (S 33.118), which had been refed six months after maintenance from three to twenty weeks of age. This rat, however, had a body weight of 229 grams, and had nearly ceased to grow. In general, therefore, we may conclude that after one week or more of refeeding the rate of mitosis corresponds in general to that of normal younger rats of similar body weight.

In these refed rats, however, marked individual variations in the number of mitoses are found, both in different individuals and in different sections of the hypophysis of the same individual. These variations usually have no obvious cause. In one very exceptional case, however, an apparent cause was found. In rat St 11.46 (table 5 C 4), refed four weeks after maintenance from three to twelve weeks, an astonishing number of mitoses was found in the pars anterior, 107 being counted in one section and 112 in another. This enormous rate of mitosis was apparently somewhat evenly distributed throughout the lobe, though no actual counts were made in other sections. It seemed to involve all types of cells. No mitoses were found in the partes intermedia and nervosa. The apparent cause of this abnormal mitosis was found in a small inflammatory lesion near the center of the anterior (distal) lobe, which presented a circumscribed area filled with polymorphonuclear leucocytes. The stimulus from this lesion doubtless caused the proliferation of cells throughout the anterior lobe, while apparently not affecting the remainder of the gland.

CHANGES IN HISTOLOGICAL STRUCTURE

The normal histology of the hypophysis in the rat has been described briefly by Tilney ('11, '13) and by Stendell ('14). In the following description, only those features especially concerned in the changes during inanition will be considered.

a. Pars nervosa

In the newborn rat the pars nervosa in structure resembles a vascular mesenchyme, with numerous stellate or spindle-shaped (neuroglia) cells, whose cytoplasm fades off into a very fine intercellular network (neuroglia fibrils). The nuclei are rounded distinct, and moderately chromatic. In some cases they appear naked and nearly free from cytoplasm, which is the typical condition from the age of one week onward.

In later stages (3 weeks and above) the nuclei become progressively scattered, usually rounded or elliptical in form, and vesicular in appearance, with average diameter of about 6 μ (range 4 to 8 μ). The internuclear mass presents a fine plexus of (neuroglia) fibrillae, interspersed apparently with a granular matrix or ground substance. Among these granules there is found a variable number of spherical masses, sometimes exceeding the nuclei in size, and resembling 'colloid' in appearance. They doubtless correspond to the colloid masses described by Herring ('08) and Trautmann ('09) in the neural lobe of various animals.

The only change noted in the structure of the pars nervosa during inanition (either in young or adults) is a variable degree of hyperchromatism in the nuclei. In extreme cases, the nuclei rarely become somewhat irregular, shrunken and pycnotic. No definite change was observed in the internuclear mass, in the fibrillae, granules or 'colloid' balls.

b. Pars intermedia

The pars intermedia (fig. 1) forms an epithelial plate separating the pars nervosa from the residual lumen (hypophyseal cavity). This plate is only a few cells thick in the central region, but thicker peripherally. The cell boundaries are usually indistinct. From the age of three weeks onward the cells of the limiting layer (next to the lumen) are more or less flattened, and rarely present small ciliated areas.

In structure, the cells usually present cytoplasm filled with fine, pale violet granules, resembling those of the faintly basophilic cells of the pars anterior. The nuclei are round or oval, and moderately chromatic. Hyperchromatic and even pycnotic nuclei are occasionally found, but are rare in the normal animals. They are mentioned by Stendell ('14) as of uncertain significance.

During inanition, the pars intermedia presents a varied structure, but on the whole the changes are usually not very marked. These changes are somewhat similar in the young rats held at maintenance, and in the older rats subjected to acute and chronic inanition.

The cytoplasm during inanition as a rule does not appear less abundant than in the normal animal, except in certain atrophic areas of variable size and number. Here the cytoplasm may be scanty, and the nuclei closely packed. In structure, however, the cytoplasm usually appears altered, more rarefied and sparsely granular in appearance. Sometimes it assumes a markedly reticular or vacuolated appearance, but this is not constant. Around pycnotic nuclei, the cytoplasm usually assumes a more dense and homogeneous appearance, and stains more deeply basophilic.

The nuclei of the pars intermedia cells during inanition, according to the measurements above given (table 4) show a slight decrease in size, though frequently with but little evident change in structure. There is usually a definite tendency to hyperchromatism, however, less marked in some cases but distinct in others (especially when the inanition is extreme). In some cases, especially in the atrophic areas above referred to, the nuclei present variable degrees of pycnosis. Karyorrhexis and karyolysis are rare.

In this connection may be mentioned the 'colloid.' The colloid-like masses in the pars nervosa have already been considered. In the rat, colloid does not occur in the pars intermedia, but is usually found in the lumen of the hypophyseal cavity, at and after the age of three weeks. The amount is small, though somewhat variable. It presents marginal vacuoles resembling those in the colloid of the thyroid follicles. Trautmann ('09) mentions vacuoles in the colloid of the hypophysis of domestic animals, but considers them artefacts. If these vacuoles are interpreted as secretion phenomena, it is of interest to note that in the rat they appear more typically and frequently on the surface toward the pars intermedia, more rarely on the surface next to the pars anterior. No constant changes in the amount or structure of the colloid (either in the cavity or in the pars nervosa) were observed in the rats subjected to inanition.

c. *Para anterior* (*distalis*)

The parenchyma cells of the pars anterior present the well known types (figs. 2 and 4). These have been classified in various ways, the most useful for present purposes perhaps being that of Trautmann ('09). He recognizes the following classes of cells: (1) acidophilic (strongly or weakly); (2) basophilic (strongly or weakly); (3) chromophobic. The number, arrangement and structure of these cell-varieties differ considerably in different species; and, at least in the rat, in different individuals.

The chromophobes form an undifferentiated type of cell, relatively numerous in the newborn rat, but somewhat rare after the third week. The weakly basophilic type is usually the most numerous, the strongly basophilic rare. The acidophilic (eosinophilic) type is evident in the newborn and at three weeks, though much better differentiated later. It usually forms at least one-third of the total number of cells. The weakly acidophilic are more numerous than the strongly acidophilic, though it would be hard to draw the line between them. In fact, there appear to be numerous transition forms between all varieties (especially the chromophobic and weakly basophilic).

Tilney ('11) describes the basophiles as occupying the periphery, and the acidophiles the central region. I do not find any constant arrangement of this sort. The acidophiles are usually scattered throughout the lobe, either singly or in small groups, interspersed with basophiles. Sometimes, however, either basophiles or acidophiles may predominate in the periphery.

In the normal gland, the cytoplasm of the chromophobic cells is very small in amount, sparsely granular, and faintly staining (fig. 2). In the basophilic and acidophilic types, the cytoplasm is more abundant and usually finely (sometimes coarsely) granular in appearance. In the strongly chromophilic cells, however, the cytoplasm tends to become more homogeneous in appearance. In the eosinophiles, at least, this is apparently because the stain affects the intergranular substance as well as the granules of the cytoplasm (figs. 2 and 4). Sometimes the cytoplasm presents a finely vacuolated appearance. Various developmental stages of the 'ring-cell' type described by Addison ('16) are occasionally met in rats of ten weeks or more. The cell-boundaries of the acidophiles (eosinophiles) are usually distinct, while those of the basophiles and chromophobes are indistinct. The various types of cells in the hypophysis of the normal rat are well shown by colored figures in the second volume of Biedl ('13).

The nuclei of the chromophobic and weakly chromophilic cells are spherical or ellipsoidal in form and similar in structure, presenting a distinct nuclear membrane and an indistinct, irregular nuclear reticulum with one or more distinct karyosomes (figs. 2 and 4). The nuclei are somewhat vesicular in type and only moderately chromatic. To a certain extent, the amount of chromatin appears to vary inversely with the age, since the nuclei stain more deeply in the younger stages (up to three weeks) than in the older (ten weeks or more).

The nuclei of the eosinophilic cells also show a tendency to more highly chromatic (deeply staining) condition, which is especially marked in the strongly eosinophilic (as noted by Stendell '14), as well as in the rarer strongly basophilic cells. In the strongly eosinophilic cells, it was frequently noted that the nuclear matrix (karyolymph) becomes acidophilic, staining red like the cytoplasm. It may likewise appear purplish in the strongly basophilic type.

In a few of the strongly chromophilic cells the nuclei may in extreme cases present even a pycnotic condition. This tendency to pycnosis is usually more evident toward the periphery, near the surface of the gland and usually involves more or less atrophy of the cytoplasm. In addition to these apparently normal pycnoses, others may be observed in the region of injuries produced during removal of the gland. This was found also in the thyroid gland (Jackson '16). Pycnotic nuclei, possibly degenerative in character, were observed by Schönemann ('92) in the pars anterior of the human hypophysis, and by Stendell ('14) in the rat.

During inanition, the changes in the parenchyma cells of the pars anterior are similar in the young rats held at maintenance, and in older rats subjected to acute and chronic inanition. As might be expected, the changes are usually more marked in cases where the inanition is more protracted or severe. The changes vary greatly even in different parts of the same individual, so that great care is necessary in drawing generalized conclusions. Some areas may even remain nearly normal in appearance, while others show extreme changes of atrophy and degeneration. In general, such changes are usually found more marked and extensive in the peripheral portions of the gland. The surrounding pressure might render the surface layers of an organ somewhat more prone to atrophy, but it is difficult to understand why this should be so pronounced in the case of the hypophysis.

As already shown, there is during inanition usually a marked loss in the volume (both relative and absolute) of the cytoplasm. Even in areas retaining a considerable amount of cytoplasm, its structure frequently becomes coarsely vacuolated. In other cases (especially during maintenance and chronic inanition) these vacuoles apparently coalesce to form a watery intercellular substance, leaving the nearly naked nuclei surrounded by a thin layer of cytoplasm (fig. 3). On account of such changes, together with the hyperemia characteristic of inanition, it is evident that the loss in protoplasmic substance may be (and usually is) proportionately much greater than the loss in gross weight of the gland.

The cytoplasm also tends in general to lose its specific staining reactions. The strongly chromophilic types stain more faintly, and the weakly chromophilic become chromophobic. In extreme cases, and in areas of marked atrophy, all cells may become chromophobic, with no trace of acidophilic or basophilic reactions. For the most part, however, the cytoplasm does not reach this stage, but becomes more or less sparsely granular, with a corresponding diminishing intensity of the specific stain. In some regions (especially in the central portion of the gland) the staining reactions may be preserved much better than elsewhere.

Corresponding to the cytoplasmic atrophy, there are also changes in the nucleus. That there is loss in volume (though less than in the cytoplasm) in the nucleus has already been shown by measurements. In structure, some areas may show relatively little nuclear change, but in the great majority of cases there is a very decided hyperchromatism (figs. 3 and 5). This is best marked in the peripheral atrophic regions above referred to, where all the nuclei may present the typical pycnotic condition, shrunken, deeply staining and homogeneous. Or intermediate stages may occur, in which the nuclei are larger, with the chromatin dissolved into a pale-blue homogeneous matrix which obscures the nuclear network. Karyorrhexis and karyolysis also occur, but are comparatively rare.

In cases or areas where the atrophic changes are not so far advanced, it appears that the pycnosis first becomes evident in the nuclei of the eosinophiles. This might be expected since, as above stated, hyperchromatism and even occasional pycnosis occur in the strongly chromophilic cells of the normal gland. In some cases during inanition it appears also that the acidophilic reaction of the karyolymph may persist longer in the nucleus than in the cytoplasm of the eosinophiles. This may be due to physical rather than chemical conditions, however.

d. Changes during refeeding after maintenance

Associated with the mitoses already noted in the hypophysis upon refeeding after maintenance, changes take place in the cells leading to a restoration of the normal structure, at least in the greater part of the gland.

After one-half week of refeeding, although the gland has increased appreciably in weight, and mitosis has begun in the pars anterior, the cell structure typical for inanition still persists to a very large degree. The only changes noted are a lessening of the hyperemia in the pars anterior, and some increase in the amount of cytoplasm, with perhaps a slight decrease in the hyperchromatism of the nuclei in a few places. In general, however, it would be difficult to distinguish this stage from that at the end of the maintenance period.

After one week of refeeding, however, some areas (especially in the partes intermedia and nervosa) have become nearly normal in histological structure. In the pars anterior, these areas are more frequently found in the central region, and their extent varies considerably in different individuals. In general, however, the structure characteristic of inanition still prevails over the greater part of the gland.

After two weeks of refeeding, the normal structure becomes progressively more evident, and usually prevails over the greater part of the gland. The cytoplasm and nuclei become nearly normal in size and structure. Both pars intermedia and pars anterior still retain atrophic areas, however, especially in the periphery of the latter.

Even after four weeks or more of refeeding, although the gland in general has usually become nearly normal in structure, some more or less atrophic areas may still persist. These are much more frequent and extensive than the similar areas previously mentioned as occasionally found in the normal gland, which usually involve only single cells or small groups.

The recovery of the cells upon refeeding apparently depends upon the extent of degeneration (especially of the nucleus) involved during the inanition period. It is probable that nuclei in the extreme stages of pycnosis are beyond the possibility of repair, although they may persist in this condition for a long time before disintegration and removal.

DISCUSSION AND CONCLUSIONS

The effects of inanition upon the hypophysis were studied by Guerrini ('04). He found in the hypophysis (anterior lobe) of 4 dogs, 4 rabbits and 4 pigeons, during the first third of the acute inanition period, a slight increase of secretory activity in the cells, as indicated by a more intense reaction to Galeotti's stain. In the remaining period of acute inanition, however, he found a progressive decrease in staining capacity (granules and plasmosomes) with vacuolization of the cytoplasm. The final appearances are described as follows:

> Negli animali morti di fame, uso l'espressione nel senso il più lato, le cellule sono tutte, o presso che tutte, ridotte come in vesicole, quali più e quali meno gonfie, con nucleo, anch'esso, un po' vuoto e rigonfio e il protoplasma ridotto ad un velo, interrotto qua e là di qualche vacuolo e con appena una traccia di granuli o di plasmosomi.

In several dogs and rabbits (both young and adult) subjected to chronic inanition, however, Guerrini found no apparent change in the secretion (specific staining reactions) of the hypophysis cells. Unfortunately no details are given as to the exact character and extent of the chronic inanition. Possibly his negative results may be due to the comparative mildness of the inanition. His findings in the later stages of acute inanition are in general agreement with mine, excepting that he describes the nuclei as vesicular rather than pycnotic in structure.

It is a noteworthy fact that most of the changes above described in the cells of the hypophysis—arrested mitosis (resumed on refeeding), shrinkage in cell volume, nuclear pycnosis and loss of specific cytoplasmic staining reactions—are strikingly similar to those found in the hypophysis of the hibernating marmot (woodchuck) by Gemelli ('06) and by Cushing and Goetsch ('15). Mann ('16), however, points out that if such changes are the cause of hibernation, they should appear well-marked at the beginning of hibernation, since later they might be merely a result of the long continued torpid state. He finds these changes inconstant in the hibernating gopher (Spermophilus). In view of the striking similarity of the cell changes in the two conditions (hibernation and inanition), it seems highly probable that the changes described in the hypophysis during hibernation are simply the effects of the chronic inanition involved.

Since the rapid growth of the body in young rats upon refeeding after a period of maintenance precedes the reëstablishment of the specific cell-granules (acidophile or basophile) in the hypophysis, it is evident that their function can not be the cause of the body growth. It is, however, at least theoretically, possible that the absence of cell-granules might indicate a hyperfunctional condition, in which the granules are absorbed too rapidly to allow them to form the normal accumulation in the cytoplasm. Moreover it should be remembered that the granules are rarely absent altogether. In most cases they are found even in extreme inanition, though in scattered areas and more or less reduced in amount. It is likewise evident that the 'colloid' can scarcely be considered as the functional cause of growth, for, although it persists apparently unaffected by inanition, it is normally absent in the very young animals, in which the growth rate is more rapid. It may indeed be recalled that the most rapid growth of the body in all cases occurs in the early embryonic period, preceding any differentiation whatever in the hypophysis.

So far as the hypophysis is concerned, therefore, no evidence appears in favor of the suggestion by Osborne and Mendel ('16) that the accelerated growth following periods of suppression may be due to specific histological changes in the ductless glands. Likewise no such changes were found in the thyroid and parathyroid glands (Jackson '16). There is, however, some evidence in favor of the view that the rapid growth upon refeeding is due to the embryonic type of structure produced by the inanition in the cells of the body in general (Stewart '16). The nuclei become relatively larger and richer in chromatin, the cytoplasm small in amount and undifferentiated in structure. According to the theory of Minot ('07), these are the characteristics upon which the more rapid growth of embryonic cells depend.

While the cell changes produced by inanition may in general facilitate rapid growth upon refeeding, when pushed to the extreme (as above shown) the cells degenerate to such an extent that recovery appears impossible. This is in agreement with the generally accepted doctrine that severe inanition in young animals may produce permanent stunting of body growth; although Osborne and Mendel ('16) obtained no permanent stunting by long periods of growth suppression in the albino rat.

SUMMARY

1. During normal postnatal growth there is considerable individual variation in the relative volumes of the three parts (lobes) of the hypophysis; but in general in the older rats the pars anterior (distalis) becomes relatively larger, and the pars nervosa correspondingly smaller, the pars intermedia remaining about the same in relative (percentage) volume.

2. The relatively larger hypophysis of the female rats is due chiefly (if not entirely) to a larger anterior lobe.

3. During inanition, the volume-changes in the lobes are variable. In young rats held at maintenance (constant body weight), the pars anterior is somewhat reduced, the intermedia and nervosa correspondingly larger. In chronic (adult) inanition the partes anterior and intermedia appear reduced, the nervosa increased. In acute (adult) inanition, the pars anterior appears relatively increased, intermedia decreased, and nervosa unchanged in relative volume.

4. In young rats refed one-half week, one week, two weeks and four weeks after maintenance, there is some variability, but in general a gradual return to the normal proportions in the lobes of the hypophysis. After a prolonged period of maintenance, however, the relative volume of the lobes may remain permanently abnormal.

5. In the pars anterior of the normal newborn rat, the vessels and associated stroma form 6.7 per cent by volume, increasing to 9.6 per cent at three weeks, and to 10.6 per cent at ten weeks (adult condition). In young animals held at maintenance, the volume of the vascular stroma usually increases to about 13 per cent, and in acute or chronic inanition of adults to about 17 per cent. The parenchyma is, of course, correspondingly reduced in relative volume.

6. In the parenchyma of the pars anterior the nuclei form about 34 per cent of the total cell volume in the newborn, decreasing to about 24 per cent at three weeks and to 20 per cent at ten weeks (adult relation). The cytoplasm increases correspondingly in relative volume. During inanition, the loss is usually greater in the cytoplasm, the nuclei thereby increasing

to 26–28 per cent of the cell volume in the young held at maintenance, and to 23–26 per cent in adults with chronic or acute inanition.

7. According to data obtained by the volumetric method, the (calculated) average diameter of the parenchyma cells of the anterior lobe increases from 10.1 μ in the normal newborn to 11.9 μ at three weeks and 13.6 μ at ten weeks (adult condition). In young rats held at maintenance, the average cell diameter is reduced to 9.7–10.2 μ; in starved adults to 10.8–11.0 μ. The nuclear diameter averages 5.9 μ in the normal newborn, 5.8 μ at three weeks, and 6.0 μ at ten weeks. In the young rats at maintenance the nuclear diameter is reduced to 4.9–5.3 μ; in starved adults to 5.3–5.5 μ. Direct measurements by another method (with filar micrometer) gave similar results for the nuclear diameters, including also those of the pars intermedia.

8. The number of mitoses in an entire section of the gland is quite variable. Amitosis was never observed. In the normal newborn pars nervosa, the average number of mitoses is 7 per section; at seven days they are rare, and none occur later. In the pars intermedia, the average number decreases from 9 per section in the newborn to about 1 at three weeks; at ten weeks and later they are rare. In the normal pars anterior the rate likewise decreases, being about 62 at birth, 18 at one week, 7 at three weeks, 2 at ten weeks, and rare in adults.

9. In young rats held at maintenance from three to ten weeks of age, mitosis has nearly ceased. No mitoses were found in the partes nervosa and intermedia, although in the pars anterior they still occur occasionally, even in rats nearly dead from inanition. No mitoses were observed in the starved adults.

10. In the young rats refed after the maintenance period, mitoses reappear promptly in the pars anterior, the average number per section being about 2 after one-half week of refeeding, 7 after one week to two weeks, decreasing to an average of 3 after four weeks of refeeding. Mitoses were observed but rarely in the pars intermedia, and never in the pars nervosa. The rate of mitosis in the hypophysis of the refed rats therefore

corresponds roughly to that in younger normal rats of similar body weight.

11. In cell structure, the only change noted in the pars nervosa during inanition is a variable degree of hyperchromatism in the nuclei, which rarely may become shrunken and pycnotic. In the pars intermedia, most of the cells usually suffer relatively little change during inanition. The nuclei have a variable tendency to hyperchromatism, occasionally becoming pycnotic, especially in certain atrophic areas. The cytoplasm tends to lose its granular structure, becoming more homogeneous and often finely vacuolated in appearance. Around pycnotic nuclei it is usually more strongly basophilic, and is much reduced in volume in the atrophic areas above mentioned.

12. The colloid which occurs normally in the pars nervosa and in the hypophyseal cavity (residual lumen) appears unaffected by inanition.

13. In the pars anterior, the changes during inanition are quite variable. Some areas may remain nearly normal, while others, even in the same gland, show extreme changes of atrophy and degeneration. The cytoplasm is usually reduced in volume (as above shown) and is frequently much vacuolated. The structure becomes sparsely granular and there is a marked tendency to loss of the specific staining reactions, so that the strongly chromophilic cells become weakly chromophilic or even chromophobic. The nuclear changes are likewise variable, but there is a very general tendency to hyperchromatism, often reaching a definite pycnosis. Karyorrhexis and karyolysis are rare.

14. Upon refeeding one-half week after the maintenance period (three to twelve weeks of age), the hypophysis still retains the typical inanition structure, although mitosis and growth have begun. After one week of refeeding, some areas have become nearly normal, and after two weeks the normal structure preponderates. After four weeks, the greater part of the hypophysis appears nearly normal, although atrophic areas may persist for indefinite periods. Recovery is improbable in cells whose nuclei have reached advanced pycnosis.

15. The changes described in the hypophysis during hibernation are probably inanition effects. The rapid growth of the body following periods of inanition may be due to the embryonic condition to which the cells are reduced; but there is no evidence that such growth is due to any specific histological changes in the hypophysis or other ductless glands.

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今回出版ノ米國解剖學雜誌特別號ハ特ニ日本號ト命名シ通常號ト異ナリテ論著ノ本文ノ外ニ一々著者自抄ノ摘要ヲ日本文ニ譯出シテ掲載仕候 本號發行ノ目的ハ日本ニ於ル生物學上ノ研究ニ敬意ヲ致シ尚一層解剖學ノ進運ヲ希フ米國同學者ノ切望ヲ表スルモノニ外ナラズ之ニヨリ日米兩國學者間ノ交誼ヲ助クルヲ得バ喜悦不過之候 洋ノ東西相携ヘテ人道ノ爲人智ノ啓發ニ向テ一致協力シテ盡瘁センコトハ本研究所ノ衷心ノ希望ニ有之候

大正六年五月

米國費府ウィスター解剖學及生物學研究所

血節ノ研究　（其六）牛及山羊ノ血節

マイヤー　述

著者ハ本題ニ關スル文献ヲ簡叙シ、血節ノ位置、數、大サニ就テ論述シ、特ニ皮下血節モ常ニ血管系ニ關聯シテ存スル旨ヲ細述セリ。牛ノ血節ハ淋巴節ノ在ル場所ニハ決シテ在存セズ。腹腔内ノ血節ノ多クハ小ナルモ皮下ノ血節ハ大形ニシテ多クハ肩及側腹部ニ限局セリ。本研究ノ材料ハ各齡ヲ網羅セリ。

山羊ノ血節ハ概シテ少數且小形ナリ。皮下血節ハ存在セズ、又生活細菌ノ注射又ハ反復瀉血ヲ行フモ淋巴液ガ血節中ニ入來ルコトナシ。混合型ノ節モ見出スヲ得ザリキ。本研究ニハ山羊仔數頭及成熟セルモノ凡十二頭ヲ用ヰタリ。

STUDIES ON HEMAL NODES

VI. HEMAL NODES IN BOVINES AND GOATS

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ONE FIGURE

Since the statements in the literature regarding the hemal nodes of bovines and of goats with one exception, are extremely brief a short historical review seems not only justifiable but advisable. Besides giving due recognition to the observations, conclusions and opinion of others it will also afford a better basis for independent judgment.

The first definite statement regarding hemal nodes in bovines was made by Robertson, '90 who declared that the hemolymph nodes of the bullock are larger than those of the sheep and that they vary in size from a mustard seed to a pea. Robertson also found the trabecula larger and broken up in some instances, by small blood sinuses. The centers of the follicles were also found to contain more 'pale' cells but Robertson concluded that the hemolymph gland of the bullock is formed on the same plan as that of the sheep and differs from it in no essential detail.

Vincent and Harrison, '97, found "large numbers of blood-red bodies distributed irregularly on either side of the vertebral columns" and also for some distance in the pelvis. But they found only a few nodes in the thorax near the roots of the lungs and in the connective tissue of the mediastina. These investigators found the capsule of hemolymph nodes varying widely in thickness, containing leucocytes and occasionally excavated by sinuses. The latter were lined by endothelium which "was reflected over the trabeculae in the sinus." They also called attention to the fact that some of the hemolymph nodes which lie near the kidney and renal vessels look more like lymph glands and concluded that hemolymph glands are modified lymph glands and develop from them.

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Drummond, '00 found hemolymph nodes from 0.5 to 0.75 mm. in size on the posterior abdominal wall of a foetal calf 9 inches long. On section these were found to be well-formed hemolymph nodes which contained erythrocytes in the capillaries and also in irregular spaces found mainly near the hilus and in the center of the gland. In order to judge better what Drummond was actually describing it is well to recall that he found the lymphatic tissue of hemolymph nodes usually divided into a cortex and medulla and the generally abundant germ centers arranged in two rows.

Lewis, '03, who merely referred to Bos taurus, reported that he found endothelial cells containing pigment in the hemal nodes of the ox and declared that hemal and hemal lymphatic glands "are distributed with considerable constancy in three main groups: renal, splenic and subvertebral."

White, '04 found that "very little difficulty is experienced in identifying from gross appearance hemolymph nodes in the ox, sheep, pig, etc.," but no description of the nodes is given in the report and from the above statement one is compelled to conclude that White probably misapprehended the real situation.

Crescenzi, a government inspector at a public abattoir, in '06 was the first to report the presence of hemal nodes under the skin in Bos taurus but, strangely enough, did not attach any special importance to this fact or describe these nodes in particular. Moreover, Crescenzi emphasized the fact that hemal nodes are always found near lymph nodes and mentioned the pelvis and the peribronchial and perirenal regions as sites of predilection. Not rarely he also found them under the skin and pleura and between the muscles of the extremities. In the former locations their form which generally tended to the spherical, was somewhat flattened on account of lateral pressure.

Crescenzi found hemal nodes somewhat more numerous in cattle from Sardinia and particularly abundant in calves, lambs and kids. He failed to find them in young foetuses but counted ten nodes in each of four foetuses from seven to eight months old. These nodes varied in size from the head of a pin to a "grain of pepper" and many of them were in contact with lymph nodes from which it was difficult to separate them. Their general histological appearance was intermediate between that of hemal and lymph nodes, after birth.

Crescenzi also found hemal nodes imbedded in the fat of the hilus and also on the surface of lymph nodes. An iliac node of the size of an almond was said to contain nine independent hemal nodes varying in size from a "grain of pepper to a hazelnut," in a deep dilatation of its hilus! Another iliac node had three hemal nodes varying in size from the "head of a pin to a pea," partly imbedded on its free surface.

Carcasses containing an unusual number of hemal nodes were also found. One of these carcasses contained a large intra-abdominal abscess as large as a child's head, in a second a splenic tumor was present, while in a third the spleen was atrophic and sclerotic. Crescenzi also emphasized the fact that lymph nodes may contain so much blood especially in their darker portions—as was the case in the ox with a large abdominal abscess "(slaughtered for the royal marine)" that they are indistinguishable from hemal nodes and concludes with the words "E se tale si mette in rapporto colla quantitat veramente eccezionale di gangli ematici riscontrati se ne puo dedure una relazione funsionale."

In July '07 Piltz also reported the occurrence of lymph nodes of reddish color in the subcutaneous tissues and in part also beneath the panniculus carnosus in cattle. According to Piltz a portion of these nodes was described by Martin as those of the 'Hungergrube.'[1] Piltz also reported the finding of subscapular and aural hemolymph nodes near the corresponding lymph nodes. He also found from eight to ten nodes in the subcutaneous tissues located caudal to the ligamentum axillaris near the vena thoracalis lateralis, on the dorsal border of the subscapularis and under the panniculus carnosus over the 'Hungergrube' and on the tubera ischii, on the costal arch and on the neck. Piltz found the hemolymph nodes of emaciated cattle somewhat larger but normally seldom larger than a pea although some nodes found near the deep cervical nodes were said to equal the size of a hazelnut. Small nodes were also found distributed irregularly in the pelvis and also a few in the thorax along the course of the aorta as far cranially as the bifurcation of the trachea, but occurred more frequently on the left side of the pericardial mediastinum. Although Piltz found the thoracic nodes near the tracheal lymph nodes most constant and also found nine small nodes near the larger popliteal lymph nodes, he did not think that hemolymph nodes are limited in their location to that of lymph nodes. Piltz found hemolymph nodes to vary much in their content of blood and concluded that they assume the function of the spleen after splenectomy.

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[1] Although it is very evident what is meant by this designation and why it is used I have been unable both upon inquiry and by consultation of texts on veterinary anatomy to find an equivalent term in English for this very prominent fossa. Hence I suggest the term pre-iliac.

One month later Baum, '07 reported the results of an examination of nine bovines and two sheep. Baum found hemolymph nodes on the visceral surface of the liver—a unique instance, I believe, on the cranial portion of the trapezius and not seldom beneath the skin or the panniculus carnosus in the region of the costal margin, or near the 'Hungergrub.' But Baum emphasized particularly that hemal nodes are found chiefly near large lymph nodes, that he could not distinguish a medulla or a cortex and that all manner of transition forms occur.

In 1908 Forgeot stated that the lymph nodes of cattle are usually in connection with large afferent vessels which are filled with pink or even with red lymph which contain erythrocytes but that other glands entirely independent of the lymphatics are not rarely found in the abdominal and thoracic cavities of bovines and goats. Forgeot also stated that hemal nodes are only in connection with lymphatics during early life when they have a hematopoetic function and claimed that closed, blindly-ending lymphatics filled with blood can be demonstrated to communicate with the sinuses of the nodes.

Although Forgeot definitely stated, in 1908, that nodes wholly independent of lymphatics are found in the abdomen and thorax of cattle, Forgeot asserted, a year later, that injections are wholly unnecessary *to demonstrate the existence of afferent and efferent* *lymphatics* in the hemolymph glands found in the neighborhood of the aorta and inferior vera cava in goats and cattle. Forgeot also found nodes in the lumbar region in the sheep and goats and beneath the pericardial pleura in cattle, from which blindly-ending lymphatics extended. These lymphatics were represented and described as shorter or larger, markedly distended tubes which lay in the surrounding connective tissue and returned to the node. A network of lymph vessels filled with 'red lymph' was also seen around some hemolymph nodes. Forgeot concluded that hemolymph nodes develop independently of the pre-existing lymphatics and that lymph vessels join them only secondarily.

In 1908, I independently reported finding subcutaneous hemal nodes in bovines giving their location, number and size, etc., and stated that I was unable to demonstrate the presence of lymph vessels by means of injections of India ink made directly into the nodes on carcasses of full grown cattle. I then traced these nodes in foetuses down to a V. B. length of 22 cm. In younger foetuses, I was unable to identify them. That report also contains a comment on their structure and on some other matters.

The only full statement regarding hemal nodes is found in the thesis of Piltz, '10. Although Piltz does not indicate the scope of his examination he says that he used India ink and blood serum to inject the *mesenteric* glands and vessels of *ill*-fed bovines. (The italics are the writers). Among other matters to be referred to in connection with my own results, Piltz stated that his results are essentially like those of Weidenreich on sheep. Piltz also stated that although he had no convincing proof for the assumption, yet he considered hemal nodes only a stage in the development of lymph nodes and declared that he found sufficient indication for the constant neo-formation of hemal nodes and of a constant readjustment within the nodes themselves. However, Piltz presents no evidence whatever for these opinions, except to suggest that certain nodes which are almost wholly depleted of lymphatic tissue are in a developmental stage and to point to the existence of mixed forms of nodes. Since Piltz found these mixed forms; that is nodes a portion of which contained mainly blood and the rest only lymphatic tissue; in animals several years old he assumed a continued new formation. According to Piltz's conception lymph vessels grow into the node after the sinuses (sic) have been replaced by lymph cells and the node has been converted into a lymph node. Piltz also regarded the blood sinuses as dilated capillaries *originally*, i.e., during early development—but nevertheless thought it unlikely that hemal nodes were ever in connection with the *vascular* system!

Tixier and Duval, '10 also described, in vaccinated calves, certain glands which had the general structure of lymph glands but which possessed no lymphatics. They found four, eight or ten or even more, dark reddish nodes imbedded in the thymus or in contact with its cervical or cardiac portions or within the mediastinum. It is significant, however, that these nodes reminded them of similar nodes found previously near the thymus in infants, and that those from the vaccinated calves contained sinuses exactly like those in lymph nodes but larger. These sinuses the authors considered as the beginning of veins and surmised that the arteries open directly into the lymphoid tissue.

Schellhase, '11 reported that hemolymph nodes are commonly present in bovines, sheep and goats in the tropics and reported two cases in zebus in which innumerable nodes were found in the interstitial tissue of the lungs. In one case these nodes varied in size from the "head of a pin to a lentil" while in the other, they were as large as a bean. Their structure was said to be typical—whatever that may mean—of hemolymph nodes in all cases.

The occurrence, location and structure of hemal nodes of the domestic sheep, of guinea pigs, rabbits, cats, dogs, goats, et cetera, together with the occurrence and alleged experimental production of supernumerary spleens were discussed in the previous papers of this series (Meyer, '13 and '14). Hence the present discussion will be limited to two species except in so far as evidence obtained in the others helps to elucidate some of the special features.

The material upon which this article is based was far less extensive than in case of the cat, dog, and especially the sheep. Nevertheless, hundreds of carcasses of beeves were inspected but injections were made only on the subcutaneous and abdominal hemal nodes and the microscopical examination was confined mainly to foetal and adult specimens of subcutaneous nodes. The latter are particularly accessible and can be examined *ad libitum* in abattoirs without damage to or soiling of the carcasses, while the lumbar or even the abdominal nodes as a whole, are generally accessible only with difficulty because they are so deeply imbedded in fat. The purchase of living animals did not seem advisable or justifiable.

Since the nodes in goats are as easily accessible as those in sheep no especial difficulty is encountered in them except for the comparative paucity of the nodes and the fewness of the carcasses of goats in abattoirs. Hence only six carcasses and two animals—all adults—were used. Two of the six carcasses were from Angora goats.

The most striking contrast between the hemal nodes of bovines and the sheep lies in the larger size and comparative fewness of the lumbar, and the greater number of the mesenteric and thoracic hemal nodes, and especially in the presence of large subcutaneous hemal nodes, in bovines. Moreover, apparently mixed forms, that is nodes partly dark red and gray and of unusual size, also seem far more numerous in Bos taurus. I take it that Lewis '04-a saw these when he said that "Many of the largest glands (9-10 cm. long) have not this structure, but are of the nature of certain glands recently described by Weidenreich. (Verhandlungen der anatomischen Gesellschaft vom 22, April, 1902). The particular structure here referred to by Lewis is one in which there is a mixture of blood and lymph in the sinuses. These nodes which Lewis did not find larger than 2 by 1 cm. in ungulates, he regards as typical hemal lymphatic glands. The form described by Weidenreich, on the other hand, according to Lewis, are nodes which contain distinct and separate blood and lymph sinuses. Since Lewis declared that "The lymph sinuses occupy one side of the organ, the blood sinuses the other; they are not indiscriminately mixed together;" it does not seem at all unlikely to me that the type referred to by Lewis, which look pink upon inspection, are large reddish lymph nodes which can not be identified positively except by injections which Lewis frankly says he did not make. Hence, these nodes were likely taken for hemolymph (hemal) nodes. They are found in the position of lymph nodes and can not infrequently be seen in direct connection with the large abdominal lymphatics. Some of them are deeply red at one end, but have the color of an ordinary lymph node at the other. Moreover, Lewis stated that there often is a fair delimitation of the lymphatic and hemal areas as judged by an inspection of the cut surface of such nodes. They also have the form characteristic of lymph nodes and are, as a rule, many times as large as the true hemal nodes of sheep and goats.

The subcutaneous nodes of adult bovines are generally larger and quite unlike lymph nodes in form, though some of them simulate them quite closely in color. Practically all are circular in outline with slightly convex or flattened sides, and with uneven external surfaces. Unless found beneath the panniculus carnosus, they stand out very prominently on the dressed beef because of their size and color, in spite of the fact that they are usually quite completely imbedded in fat. In the region of the flank, they may lie very close to the regional lymph nodes.

There may be only a single subcutaneous node on a half carcass, or there may be a dozen varying from 0.5 to 1.5 cm. in size. Scores of nodes on each half carcass were never seen, fifteen were seen but once. Twelve of these were small and lay in the scapular region. They varied in color from bluish black to a bright red or pale gray and were unusually firm. The contained blood could never be expressed from them by pressure, and as stated previously (Meyer, '08), injections of India ink into a large series of them failed to reveal any connection with the lymphatics. The injection mass invariably entered the intercostal veins and then the azygos veins. These subcutaneous nodes were never seen restricted to the location of the peripheral lymph nodes, as stated by Crescenzi, Baum and Piltz, but were found scattered or grouped more or less in certain regions such as that of the shoulders, neck and hips.

In the abdominal cavity, the absence of such large numbers of hemal nodes as seen in the lumbar region of sheep, was particularly worthy of note and although many hundreds of beeves were inspected in the abattoirs during the last half decade, I never saw a carcass that contained as many hemal nodes as were usually seen in dozens of carcasses of sheep during the course of a few hours slaughtering. The most Crescenzi mentioned in individual bovines is nineteen.

The presence of exceedingly coarse trabeculae and a very thick, often fenestrated capsule in bovine subcutaneous nodes, are as striking as is the fine architecture of the abdominal nodes of the goat. Large streamers and whorls of connective tissue are also commonly present in the former and not infrequently, as the needle penetrates the node in the course of injections, the accompanying sound is entirely comparable to that heard when piercing cartilage. This is true even of small nodes, especially if they are pale or gray.

Not infrequently the capsule is composed of a relatively thin dense outer portion and a very broad inner meshwork of coarse trabeculae, as shown in figure 1. The large meshes of this inner framework contain erythrocytes and lymphocytes, and the trabeculae, which compose it gradually merge with the framework of the interior of the node. The capsules of some nodes also contain comparatively large veins, and occasionally some fat cells. However, Vincent and Harrison, '97 stated that "In no instance did we find any fat cells inside the capsule." Since evidences in my possession seem to indicate that the presence of fat cells, which is exceedingly common in lymph nodes of the pig and guinea pig, is determined largely by the fatness of the animals this discrepancy in results is easily accounted for.

Many portions of these extremely large firm nodes are almost exclusively vascular in structure and others exclusively lymphatic except, of course, that lymphatic vessels or sinuses were never seen. In fact, the lymphocytes not infrequently are so closely packed in the lymphatic areas that scarcely any vessel could be seen in them. Many of the portions also contained follicles which were densely crowded together directly beneath the capsule or along some of the large trabeculae. The absence of fat in hemal nodes is striking.

All of the foetal subcutaneous nodes examined microscopically in 1908 contained no lymph vessels whatever but some of them contained a more or less continuous empty subcapsular space or sinus. It is obvious that the nature of this space could have been open to a double interpretation were it not for the fact that all of the subcutaneous hemal nodes of adult bovines had been found to be in connection with the veins only. To be sure, this fact does not necessarily exclude the questionable possibility assumed by v. Schumacher '12, that lymphatic vessels may grow into nodes and then disappear only to re-enter the nodes later.

Some of them contain practically no extra-vascular erythrocytes. Many foetal nodes look red to the naked eye, however, because the blood vessels are relatively large. But the most striking thing about these minute foetal nodes, is the presence of large numbers of giant cells, some of which were of considerable size. From two to six of these cells could be seen in almost any section, and several were not infrequently grouped in a small area. These poly- and megakaryocytes were especially numerous in the nodes of foetuses near term. They were always somewhat irregular in outline, occasionally very much so, seldom contained inclusions and as in the case of the nodes of the sheep always lay in the lymphatic tissue. Eosinophiles were not seen in the bovine nodes examined and pigment was seen only in nodes which were practically sacs of blood.

The erythrocytes were scattered about indiscriminately among the lymphocytes in most of these young nodes and in several cases a vessel was seen opening directly into the subcapsular space thus confirming similar appearances rarely seen in hemal nodes from foetal sheep as reported some years since (Meyer, '08). I am at a loss for an interpretation of this observation except to suggest that it may be, in part, a survival of an early stage of development although veins from the surrounding tissue may enter the subcapsular sinuses of mature nodes. It may be recalled that a similar relationship was found to exist in the case of a very small supernumerary spleen from the great omentum of an adult. This spleen did not even fill the field of an oil immersion lense (see fig. 10, Meyer, Anat. Rec. 1914). As in case of the foetal nodes of the sheep so in subcutaneous nodes, there often were no definite blood spaces, while in others, portions of the developing node seemed to contain a plexus of engorged vessels with but very few erythrocytes scattered among the lymph cells. The latter type of node contained almost no giant cells, but the former many. From these and from similar observations made some time previously on nodes of the sheep, it seemed probable that the stage when the vessels came into relation to the developing node could apparently vary somewhat both as to time and character. This supposition is also confirmed by facts observed in connection with developing subcutaneous hemal nodes in bovines as reported in the accompanying paper.

In the goat the location and appearance of hemal nodes were entirely comparable to those in the sheep as Warthin, '02 b then stated, except that from the six carcasses and two adult animals examined I am compelled to conclude that they are far less numerous. Subcutaneous hemal nodes were not found. In one goat, a healthy and well-fed animal, for example, which was examined several hours after accidental death, only two flattened hemal nodes approximately 3.5 mm., in size were found directly beneath the peritoneum near the aorta in the left supra-renal region. These specimens were proven to be hemal nodes by injections and although the rest of the carcass was examined carefully, no other nodes were found.

In a second animal, which I owe to the courtesy of my former colleague, Professor Zinsser, no larger number of hemal nodes was found, although this animal had received repeated injections of living typhoid bacilli for a period of nine months. With the exceptions of a few intermissions, this animal had been given intravenous injections of one-third to seven slants of typhoid cultures in increasing doses, every five days at first, and later every ten days. The total number of doses given was twenty-six and Professor Zinsser stated that precipitin against typhoid was present, that the blood showed a very high agglutinating power (1–2000), gave a bacteriolytic titre of 1–4000 in vitro test, and also showed a slight inhibition (1–10,000). Yet only a few hemal nodes were present in this animal. In fact, they were fewer in both these goats than in the six carcasses of adult animals examined in abattoirs. Nor did the lymph nodes of this goat show any change whatever as judged by naked eye appearances. These facts are particularly significant in view of the opinion of Meek, '10 regarding the effect of toxins in the production of hemal nodes which was discussed elsewhere (Meyer, '14) in connection with the results of experiments on guinea pigs.

In the carcasses of the six other goats, a total of only about six to twelve nodes was present in the abdominal and thoracic cavities. Subcutaneous hemal nodes were never seen. All nodes were very small—1 to 3 mm.—and no so-called mixed forms were present in any animal. Trabeculae were practically absent in all small nodes and the whole architecture was an exceedingly delicate one. Only a few follicles and small quantities of intracellular pigment were present. Not a single giant cell was found and erythrophages were seen in large numbers in one specimen only. They were so large that they gave the section a striking appearance on low power magnification and many of them were so crowded with erythrocytes that they looked like a sac of blood. Drummond, '00 said the same thing about certain hyaline cells derived from lymphocytes which he said became amoeboid and phagocytic. These large hyaline cells Drummond found absent in foetal dogs, sheep and rats, and he thought that the formation of hyaline cells may suddenly begin and the destruction of erythrocytes then take place because he found thousands of phagocytes in sections containing unchanged erythrocytes.

In some sections about fifty quite well-preserved erythrocytes could be counted without change of focus in surface views of individual erythrophages in the nodes from these goats. Hence, each phagocyte must literally have contained hundreds of erythrocytes in greater or lesser states of degeneration. That these phagocytic cells were not polymorphonuclear leucocytes is beyond question. The nuclei were always vesicular with definite but few chromatic granules and but seldom were markedly irregular in outline. As in case of the few hemal nodes of the sheep, in which such a marked ingestion of erythrocytes was noticed, these phagocytic cells were apparently large leucocytes with a large vesicular nucleus, which are so common in hemal nodes although they may have been endothelial in origin. In most of these nodes dozens of eosinophiles were also scattered throughout the sections; however, the origin of the eosinophile granules through the ingestion and transformation of fragments of erythrocytes as claimed by Weidenreich does not seem highly probable to me. As a whole, then, the hemal nodes of goats were very small, the follicles were few, trabeculae were practically absent, the amount of connective tissue within the node was very small, the whole architecture very fine instead of coarse, as in bovines, and phagocytosis was marked.

Injections into the few nodes of Capra hircus, which were large enough to permit injection, showed them to be in connection with the veins. In two specimens which were only 1.5 to 2 mm. large, the communication of the vein with a narrow but continuous periphreal venous sinus—not the so-called subcapsular sinus, or blood space—which lies in the periphery of the lymphoid tissue just internal to the subcapsular blood space, was splendidly shown in serial sections. A section of one of these nodes was shown in figure 8 (Meyer '14) of a former article in this series of studies. Hence although my basis for judgment is not a broad one, I regard the circulatory and also the structural conditions of the hemal nodes of the goat practically identical with those of the hemal nodes of the sheep.

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血節ノ研究（其七）血節機能ノ發達

マイヤー　述

皮下血節ハ直接ニ間胚葉ヨリ生ジ、牛胎兒ノ顱頂ヨリ臀端迄ノ長サ二十二仙迷ノモノニ於テ始メテ發生ス。此期以前ノ胎兒ニテハ此部ハ微小ニシテ多少分化セル結節樣「じんしちうむ」性間胚葉ヨリ成ルヲ見ル。或例ニ於テハ此節ハ空腔ニテ包擁セラルルヲ見ルモ、此腔ハ淋巴系ト連ナルコトナシ。又發育初期ノ淋巴節ト關聯シテ見ラルベキ淋巴管叢モ認メラレズ。後、動脈及靜脈ガ節内ニ發育シ來リ、時ニ動脈ハ空腔ト連絡スルニ到ルコトアリ。

ワルシン及他ノ者ハ血節ガ脂肪織ヨリ生ズト謂ヒシモコハ確實ナラズ。唯、血節ガ全ク淋巴系ト發育上ノ關係ナキモノナルコトハ明カナリ。

STUDIES OF HEMAL NODES

VII. THE DEVELOPMENT AND FUNCTION OF HEMAL NODES

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FIFTEEN FIGURES

Some years since when hemal nodes were found in close proximity to the parathymus glands in the sheep, it was thought that these nodes would be particularly valuable for a study of development. Unfortunately, however, these nodes also are very inconstant in occurrence and here as elsewhere in the sheep, they are often closely associated with lymph nodes. Indeed, the greatest obstacle in the study of the development of hemal nodes is not the protean structure of the nodes of adult animals but the inconstancy in their occurrence and position. This is true not only in the adult animal but in foetuses as well, for although a large number of sheep foetuses of varying ages and also new born lambs were carefully dissected, not a single hemal node was ever found to be constant in position. The same statement holds for Bos taurus. Besides, the frequent close association of lymph and hemal nodes makes differentiation of the early stages in development naturally very difficult. To partly obviate these difficulties it was decided to trace back as far as possible in the foetal development of the sheep, the occurrence of nodes which could with some certainty be recognized macroscopically as such in the fat of the lumbar sub-vertebral region. The tissues in this region were also excised en masse in younger foetuses for purposes of microscopical study in order to determine, if possible, by means of serial sections the earlier stages in the development of hemal nodes which because of their small size cannot be recognized with the unaided eye. For the latter purpose small foetuses were also preserved in toto and then as much of the foetus as necessary removed after fixation and used for the same purpose.

It was possible to recognize macroscopically with some certainty, hemal nodes in the lumbar region of sheep foetuses 12 cm. (V. B.) long and microscopically somewhat earlier (Meyer '08). But as already stated, positive identification of the gross or sectioned specimen was, to be sure, not always possible, even in older foetuses. In serial microscopic sections it was possible, however, to distinguish masses of lymphoid tissue distinctly hemal in character in foetuses 9.8 cm. (V. B.). In all of these young foetuses there is considerable difficulty, however, in distinguishing early developing lymphatic nodes from hemal nodes because differentiating areas of mesenchyme which might be regarded as early stages in the formation of lymph nodes are frequently seen to be contiguous with others which plainly suggest early hemal nodes. This difficulty is further complicated by the fact that the lymphatics of developing lymph nodes are often filled with blood or erythrocytes or at least contain some blood. A further difficulty lies in the fact that some developing hemal nodes of other species contained no blood whatever thus suggesting a possibility of the occurrence of a non-vascular type or form of anlage independent of the lymphatic system in its early stages as held by von Schumacher, '12, Forgeot, '09, and Piltz, '01. From these things it is evident that confusion may easily occur where both types of nodes are found in the same region. For evidently a non-vascular anlage of a hemal node might incorrectly be assumed to be that of a lymph node and a vascular anlage of a lymph node that of a hemal node. However, the latter error, i.e., the designation of a very vascular portion of such a differentiating area of mesenchyme as hemal, instead of lymphatic, is not so likely to occur, because the early even if not the earliest stages in the development of lymph nodes although frequently vascular, show less intermingling of blood and mesenchyme even when the lymph sinuses are filled with blood and their development is generally considered to be preceded or at least accompanied by the formation of a plexus of lymph channels. The latter, however, never takes place in the subcutaneous hemal nodes of bovines and hence probably also not in case of the hemal nodes of the sheep. It may here be recalled that Drummond, '00, stated that ". . . . at a comparatively early stage in the development of the lymphatic structures in the embryo, the haemolymph glands are quite readily distinguishable from ordinary lymph glands even to the naked eye." von Schumacher, '12 a, however, declared that he could not distinguish the Anlage of the lymph from that of the hemolymph node. It has been the writer's experience to repeatedly mistake and excise small aberrant adrenals, small hemorrhagic areas, very vascular or injected fat lobules, a small plexus of blood vessels—especially of veins—parathyroids, small deeply pigmented spots in tissues, etc., for hemal nodes even in the adult. Indeed, there is at present, no means for identifying these various structures except by microscopical examination and injection. Hence, it is evident that positive gross differentiation between early embryonic or developing hemal or lymphatic nodes even with the aid of a hand lens must be wholly unreliable at present of course. Moreover, fully-formed hemal nodes which are so small that they can be excised and transferred satisfactorily only under magnification are frequently found even in adult animals. In the case of these minute specimens positive identification by means of the unaided eye must, to be sure, be wholly out of the question for they are unfortunately entirely too small to permit of direct injections.

Although Drummond's '00 article was accompanied by a drawing of a hemal node from a sheep foetus he said nothing about the development of these hemal nodes, but described those from a bovine foetus nine inches long. However, Warthin '02 (a and b) stated that the "New formation of hemolymph glands in adipose tissue was observed and the various stages of development traced. A change of hemolymph glands into ordinary lymph glands was also noted." According to Warthin "the new formation of these structures repeats their embryonal development and the earliest stages of development of both forms may run parallel." Moreover, Warthin added "The latter [the marrow lymph glands] form I am convinced, represents only a transition form in development of hemolymph nodes from adipose tissue and is to be regarded as a younger more embryonal gland." This opinion regarding the parallelism in early stages of development between lymph and hemal nodes was expressed previously by Drummond who, like Warthin, nevertheless asserted that hemal nodes were organs sui generis.

Warthin whose conclusions were based chiefly on observations made after splenectomy on sheep and goats, also stated that (1) the "transformation of hemolymph nodes into ordinary lymph nodes were all more marked" after splenectomy and that (2) "Tizzoni's description of their formation in [from?][1] adipose tissue is confirmed in every detail by the findings in the above cases." According to Warthin, '02 a, "Under certain conditions the stages of development are of constant occurrence in the prevertebral fat. Throughout the adipose tissue of the prevertebral region there appear to be certain especially differentiated fat lobules having a more definite capsule than the surrounding lobules and a richer capillary supply, frequently stand out more sharply from the surrounding fat. Under certain conditions [splenectomy] the capillaries of the fat lobules become dilated, the fat cells immediately about the capillaries lose their fat and form a reticular network in the meshes of which leucocytes assemble. Development of the capillaries into blood sinuses result in a hemolytic gland, obliteration of the capillaries through proliferation of the leucocytes and especial development of the lymphatics results in a lymph gland." Elsewhere Warthin '02b, writes that "There is undoubtedly a new formation of hemolymph nodes out of adipose tissue. All stages of this development may be seen. The process begins with an angiectatic dilation of the capillaries of a fat lobule, the fat cells of which become enlarged and lighter in color. At the same time the lobule becomes fairly well set off from the surrounding tissue by a thickening of its capsule. The next stage is an infiltration of lymphocytes along the walls of the distended capillaries coincident with an absorption of some of the fat, the conversion of the fat cells into reticular cells, and proliferation of the endothelium into the dilated capilliaries, dividing them up into blood sinuses." Continued lymphoid formation, development of sinuses and absorption of fat is said to lead to the fully developed hemolymph nodes or of the ordinary lymphoid glands. If the blood sinuses persist the structure of a hemolymph node is presented; if, on the contrary, the formation of the lymphoid tissue is so great as to reduce the sinuses to capillaries the node assumes the structure of a lymphatic gland.

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[1] The interrogatory is the writer's.

It is not my purpose to discuss the question of metaplasia or the conversion of such a mature, definitive structure as a fat cell into a lymphocyte or even a reticulum cell—yet it is only just to recall in this connection that the doctrine of the development of lymph glands from fat which is based largely on clinical evidences, on those obtained from pathological specimens, and on the older wholly insufficient experimental evidence obtained in one dog by Bayer '85, is to say the least, still sub judice as far as pathological and abnormal conditions are concerned and is not proven at all for normal conditions. This statement is made with full knowledge of the thesis of de Groot and the short article of his preceptor Reddingius '12, for their work does not set aside even if it calls in question; the experimental work of Vecchi '11 which confirmed and extended the experiments of Heuter '04 and Meyer '06. Moreover, the development of lymph nodes as recently re-investigated by Sabin '13, Lewis '09 and others is a totally different one than that indicated by Warthin. Yet my contention is not, to be sure, that what is true for lymph nodes must necessarily hold for hemal nodes or that a course of development normally followed, necessarily must be repeated exactly under pathological conditions as well, but merely that a development of hemal or of lymph nodes from fat is not proven.

Neither does Warthin's mode of development of hemolymph nodes from fat receive any confirmation from the investigation of Sabin '05. In this investigation which has stimulated so much fine work Sabin stated that the development of hemolymph nodes in the region of the neck and of the thoracic aorta of the pig "parallels the stages in the development of the other (lymph) nodes, except that its sinuses from the beginning belong to the bloodvessels rather than the lymph vessels. In lymphatic nodes the sinuses are made of modified veins, called lymphatics, while in the hemolymph nodes they are made of the veins themselves . . . . The hemolymph node found in the neck of the pig is from the beginning a distinct organ, different in type from the lymphatic node." According to Sabin "The hemolymph node does not occur in the neck of the pig until the embryo is about 23 cm. long." This conclusion of Sabin also agrees with my own observations on bovine fetuses. The superficial lymph nodes of these fetuses are well developed before the subcutaneous hemal nodes make their appearance. I also found the superficial lymph nodes well formed before the subcutaneous fat appears. This fat usually occurs in fetuses about 21 cms. long. Engel '09–'10 also emphasized the fact that it can be seen even macroscopically, that there is no developmental connection between the cervical fat and the cervical lymph nodes. Engel found the latter present in their characteristic locations before the fat appeared.

In an article on "The lymphatics" Sabin '07 further pictured a developing 'haemal' node from the neck of a foetal pig[2] 24.5 cm. long and stated that the haemolymph or haemal nodes of man are either red or brown in color according to their state of functional activity. They are brown when they contain pigment and red when they contain erythrocytes.

In 1911–12 Sabin stated that "Haemal glands have not been found in human embryos," and that in the earliest stage in the pig "the gland consists of a single follicle around a plexus of blood capillaries and surrounded by a sinus of bloodvessels . . . . . A group of lymph follicles makes a lymph gland; a group of blood follicles makes a hemal node."

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[2] The results obtained recently in numerous puncture injections on absolutely fresh carcasses of young and adult pigs leave me without any evidence for the presence of hemal nodes in the pig. Reddened lymph nodes are practically constant.

If I understand Sabin correctly the hemal and hemolymph nodes or glands are identical structures and begin their development in relation to a plexus of veins just as the lymph nodes do to a plexus of lymphatics, but the peripheral sinus of the former is formed from veins. However, the interpretation of Sabin's statement to the effect that hemal or hemolymph nodes begin their development exactly as lymph nodes do except that the peripheral sinus of the former is formed by veins, would also seem justified.

According to Retterer '07 the ordinary lymph gland begins its development in a hemolymphatic state, both white and red cells being formed therein. Hence Retterer regards all lymph glands as having been 'hemolymphatic glands' originally, and asserts that those who think there are two classes of glands do not even suspect that wholly without experimental interference, it is easy to convert one and the same gland into a 'leucolymphatic' or ordinary white or gray gland, or a 'hemolymphatic' or red gland by simply varying the conditions of alimentation. It would seem that the effects of starvation on lymph nodes even if transient as is probably the case, are too well known to need emphasis here. The effects of bleeding and of obstruction of the lymph and blood stream not to mention disease, are of course known still better to almost everyone.

If I understand Retterer correctly the terms lymph and hemolymph merely designate different physiological states of one and the same node, and the inability to demonstrate lymph vessels in the hemal nodes of sheep, is due merely to the practical difficulty of injecting the lymphatics. Be this as it may, to be wholly consistent Retterer should, of course, apply the same reasoning to the spleen and to supernumerary spleens.

As far as transformation of hemal into lymph nodes is concerned it follows, to be sure, that this is an impossibility unless (1) afferent and efferent lymphatic vessels grow into the hemal node; unless (2) the circulation of hemal nodes is transformed into a closed one; unless the obliteration of blood spaces and lacunae or the conversion of the latter into capillaries is effected and unless (3) the isolated erythrocytes and large areas of the same which are usually distributed about freely in the parenchyma of the hemal node are removed.

The difficulty of such an assumed transformation becomes doubly evident if the occurrence of very large numbers of hemal nodes which represent practically nothing but a sack of blood is borne in mind. Moreover, if such a transformation did occur, it would seem exceedingly strange that some carcass among the many thousands examined did not have a number or a group of lymph nodes in the fat of the sub-vertebral or subcutaneous regions, or elsewhere, at least approximately equivalent to the number of hemal nodes commonly found there. It would also seem strange that some hemal node or nodes with afferent lymphatics in various stages of entrance or penetration into the nodes were not found among the many, many hundreds of nodes examined macroscopically and microscopically. Furthermore, if lymph nodes can be transformed into hemal nodes some carcasses in which the customary lymph nodes in the lumbar subvertebral region had all been transformed into hemal nodes should have been found or there should, at least, have been a decided reduction in the number of lymph nodes in carcasses in which hemal nodes were so very numerous in this or in another region. Such reciprocal relations were, however, never observed and it is to be very seriously questioned whether they occur at all. Indeed, the evidences presented establishing such a transformation have been wholly inadequate, and no evidences whatever for such an assumption were observed in the course of my series of investigations which included large numbers of individuals in nine different species.

Great difficulties confront those who conclude that hemal nodes are only modified lymph nodes which may again be reconverted and thus cease to exist as hemal nodes. From a comparative anatomical standpoint it would seem strange indeed that this accident (?) in development is restricted not only to certain species and that it should predominate in certain regions of a given species, but that it occurs so much more frequently in some individuals—or at some ages—of a given species than in the case of others. Moreover, since the sub-pannicular and subcutaneous nodes of bovines previously described are always hemal and never lymphatic nodes and since the large regional lymph nodes of these animals or the sheep, are probably never converted into hemal nodes, the above assumption becomes still more improbable. Besides, if hemal nodes are converted lymph nodes and the latter converted hemal nodes as claimed by Forgeot, Piltz, Retterer, v. Schumacher and Warthin they should not only be found at the seats of predilection for lymph nodes, but the combined number of lymph and hemal nodes should not materially exceed that of the normal number of lymph nodes, unless it be assumed—which no one has done—that additional lymph nodes are formed anew simultaneously, to take the place of those converted into hemal nodes pari passu with the conversion of lymph nodes into hemal nodes.

It has not been shown that the true hemal nodes of bovines and sheep become diseased when the lymph nodes do, and no one has found a single carcass in which the place occupied so frequently by scores of hemal nodes was taken by a corresponding number of lymph nodes. The latter is, to be sure, a particularly pertinent consideration in view of v. Schumacher's conclusions and statement that hemal nodes usually possess lymphatics. Nevertheless, although v. Schumacher explicitly stated that there are two kinds of lymph nodes those with and those without lymphatics, he apparently uses the terms hemolymph nodes (Blutlymphdrüsen) and lymph node synonymously. Piltz, on the contrary, held that the blood sinuses of hemal nodes have no connection with the vascular system originally and that lymphatics only grow into hemal nodes after the blood sinuses have become obliterated by the proliferation of lymphocytes.

From embryological evidence in my hands since half a decade it seemed clear to me that hemal nodes are not in connection with the lymphatics in their early development and that the sinuses that may form in them are not in connection with lymph vessels. Furthermore, from the numerous observations on the carcasses of sheep of all ages, of several scores of the newborn lambs and of many foetuses it is evident that it is impossible to demonstrate the pre-natal or even early post natal presence of a number of anlagen comparable to the number of hemal nodes often found in the lumbar sub-vertebral region of sheep. However, this difficulty could apparently be met by v. Schumacher's suggestion that lymph nodes—among which he includes hemolymph nodes—probably continue to form in post natal life. As stated by others and also by myself (Meyer '14) this is a conclusion which it is difficult to escape in the case of both hemal nodes and of some supernumerary spleens.

Then there are, of course, the fundamental structural differences between hemal and lymph nodes which must be met by those who assume a conversion of one type of node into the other and also a reconversion into the original form or type. I previously called attention and emphasized the difficulty involved in other papers of this series. Most if not all of those who assume such easy conversions and reconversions between lymph and hemal nodes have largely overlooked or must have disregarded, the fact revealed especially by injections, that hemal and lymph nodes are distinct and separate types. This is true not only as far as the absence of lymphatics and all structural differences implied thereby are concerned, but also because of the entirely different character of the vascular circulation within the node itself. Furthermore there are other less crucial but nevertheless equally essential structural differences between the two, besides the developmental considerations here reported.

Although Retterer made no special study of hemal nodes his long series of morphological and experimental investigations on lymph nodes entitle his opinion to much consideration. Since Retterer considers lymph and hemal nodes identical a short statement of his idea of the development and to some extent of the function of lymph nodes seems pertinent. Retterer '06 found that the earliest lymph nodes in the inguinal region of the sheep appear as continuous cell masses certain elements of which become free from the protoplasmic 'font' and transform themselves at first into lymphocytes and then into erythrocytes. Their development begins as a syncytium and the germinal centers and follicular cords in all ages are composed of a syncytium the elements of which (cells, fibers and protoplasm) form a continuous whole. The erythrocytes which are later set free appear in the hyaloplasm of the reticular cells before any gangliaform enlargement is present. The production of erythrocytes does not cease in embryonic life, however, but may continue in adult life from the nuclei of lymphocytes. A full statement of Retterer's conclusions will be found in the original and in earlier articles.

von Schumacher '12b regards all hemolymph nodes as 'rudimentary forms' of lymph nodes and holds that the former always arise from the latter by a degeneration of the afferent and efferent lymphatics, either without or within the node. If the blood cells within the sinuses are subsequently destroyed then the hemolymph node is converted into an "ordinary white lymph gland without lymphatics." von Schumacher also thinks it probable that the latter nodes may as a result of sprouting of the obliterated vessels again become a true lymph node. It is of special interest that although von Schumacher holds that lymph nodes may develop wholly independently of lymph vessels he nevertheless claims that hemal nodes are not organs *sui generis*.

Rabl '13[3] found both vascular and lymphatic connections in the developing hemolymph glands (Blutlymphdrüsen) of the guinea pig and claimed that the peripheral sinus of hemolymph nodes contains blood from the beginning. It should be noted especially, however, that in the cervical node described and reconstructed by Rabl and regarded by him as a lymph node the sinus was formed from the jugular lymph sac and two veins which still communicated with the jugular vein. Hence Rabl regards the sinus as a remnant of the original communication of the node with the vein, which still carries blood to the sinus of the nodes. Nevertheless Rabl emphasizes that the only difference between his conception of the development and that of Sabin is that he regards the original vessel which communicates with the sinus and which contains blood as a lymphatic while Sabin regards it as a vein. Rabl says that the node described by him belongs in the class of hemal lymphatic nodes of the English; or 'lymphoider Bluknoten' of Baum but that he prefers to call it a 'Blutlymphknoten' in the narrower sense!

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[3] As reported in Studies on hemal nodes III, Anat. Anz. Bd. 45, 1913, I have never found a hemal node in the guinea pig.

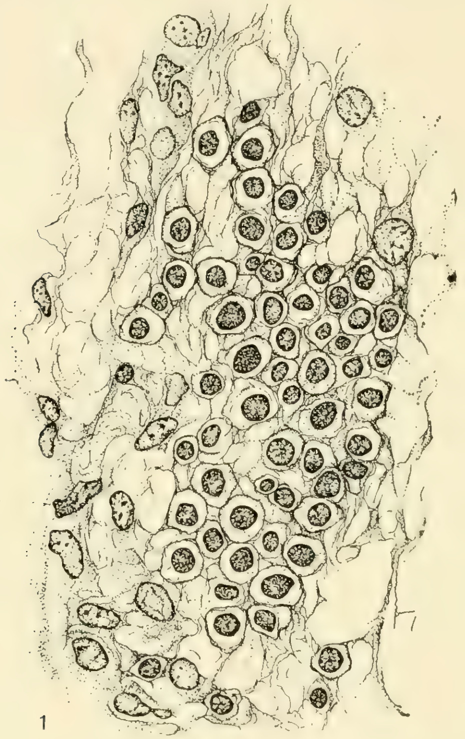
When I happened upon the subcutaneous hemal nodes of cattle (Meyer '08) and found by means of injections that these were invariably hemal and never lymphatic in character and only very rarely lay in such close association with lymphatic nodes as the hemal nodes in other parts of the body often do, it seemed that a long search for some nodes even if not for an individual node, the development of which could easily be determined was at last ended. It was, to be sure, an easy matter to trace these subcutaneous nodes back to a foetal length of 22 cm. as then reported, but unfortunately all of these foetal nodes the smallest of which were scarcely discernible with a hand lens were quite completely formed and since none of the individual subcutaneous nodes are constant as to exact position, the development of an individual specimen could not be traced by excision of the subcutaneous tissues in any small area alone. Hence, recourse had to be taken to excision of large portions of the superficial tissues in the regions in which the nodes are usually found in the adult. Although this was done in several instances at that time, nodes in the earliest stages of development were unfortunately not obtained. Because of the rather large amount of technical work involved in a rather random search and also for other reasons, the prosecution of this work was interrupted. The search for the earliest stages was continued, however, as material became available annually and extremely early stages in the development of these hemal nodes have finally been found.

As was to be surmised from our knowledge of the occurrence of the mature nodes it does not necessarily follow that the earliest developmental stages can be obtained easily by merely proceeding to younger and younger foetuses. This might,to be sure, have been the case, but since hemal nodes vary so much in the time of their appearance it so happened that the youngest stages were not found in the youngest foetuses as the legends for the accompanying illustrations will show.

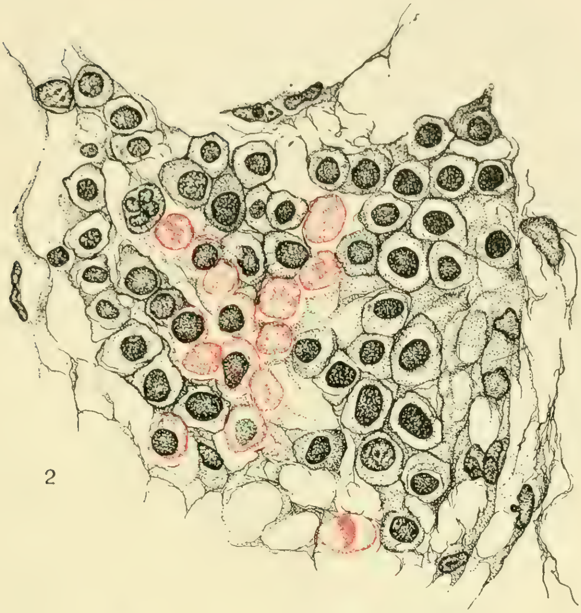
In their earliest stages the subcutaneous hemal nodes of bovines are represented merely by condensations in the subcutaneous mesenchyme. These condensations are syncytial for cell boundaries only appear later. It is true that small islands of distinct cells such as those shown in figures 1 to 3 are found in tissue from very young foetuses but these are so numerous that I seriously doubt whether they represent early stages in the development of hemal nodes. Some of them as the one shown in figure 2, apparently are blood islands rather than centers of formation for hemal nodes. Others such as that shown in figure 3 are the earliest anlagen of the fat lobules one of which in a somewhat later stage of development was found to contain a giant cell (fig. 4).

A less misleading though not uncommon appearance is the presence of a rather large mass of lymphocytes in a well-developed fat lobule as shown in figure 5. In this particular case the mass of lymphocytes is penetrated by a capillary and it is easy to see how these accumulations could suggest the formation of lymph and hemal nodes both in and from fat. Regarding the exact nature of still other accumulations like those shown in figures 6, 7 and 8 I am still in doubt. These accumulations of lymphocytes about capillaries and arterioles are never encapsulated, are not at all well circumscribed and may be cylindrical in form. From my observations on supernumerary spleens I would be willing to believe that a hemal node might arise also in this way. Indeed, it requires no stretch of the imagination or violation of morphogenic conceptions to consider figure 7 and 8 as a representation of early hemal nodes. All that is necessary is the assumption that chronological variations in the vascularization of the early anlagen occur. For such an assumption there is abundant evidence in the whole morphology of hemal nodes. Nevertheless I would not consider such a genesis as the normal or usual one and prefer not to urge it especially since such accumulations are relatively common.

The earliest anlage of a hemal node which I found is represented in figure 9. This node which is 80 μ in size represents a condensation in the mesenchyme which has become circumscribed through the development of a rather wide peri-nodal space. Although it is sometimes difficult to be certain this space is purely local and has no direct connection with the vascular or the lymphatic systems. The peripheral space which is not complete is surrounded by a slight condensation in the mesenchyme which suggests beginning capsule formation and would hence justify one in regarding this space as the peripheral sinus or as I prefer, the subcapsular space. No capillaries or erythrocytes are contained in this anlage and the cellular dif-



Figs. 1 and 2 Cell islands in subcutaneous mesenchyme. Foetuses 27 and 18.5 cm. × 1340 and 920.



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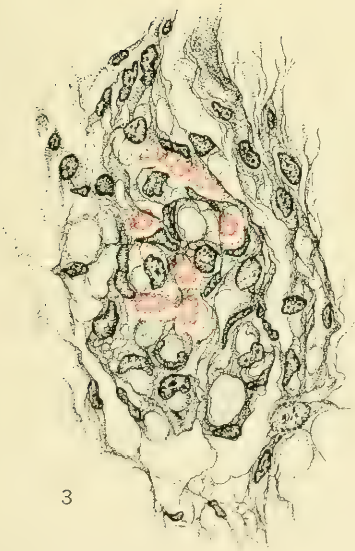

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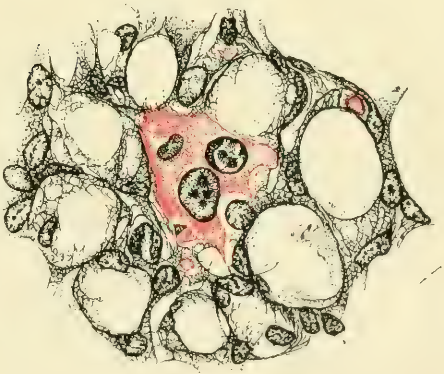

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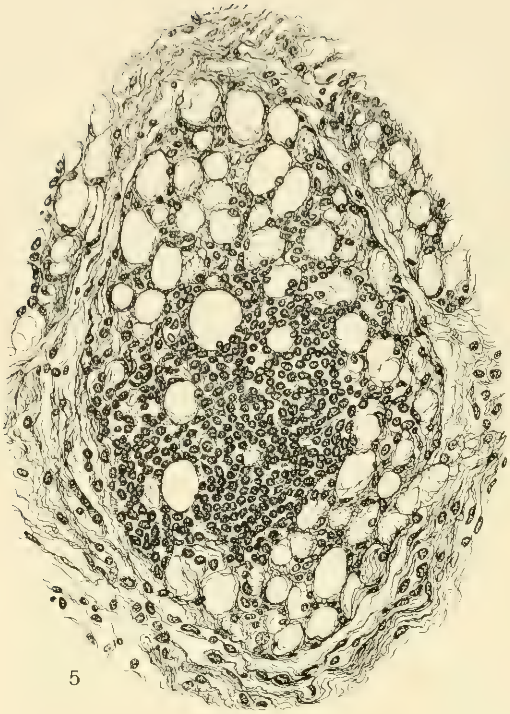
Fig. 3 Very early subcutaneous fat lobule. Foetus 31.4 cm. × 920.
Fig. 4 Giant cell in a developing fat lobule. Foetus 32 cm. × 215.



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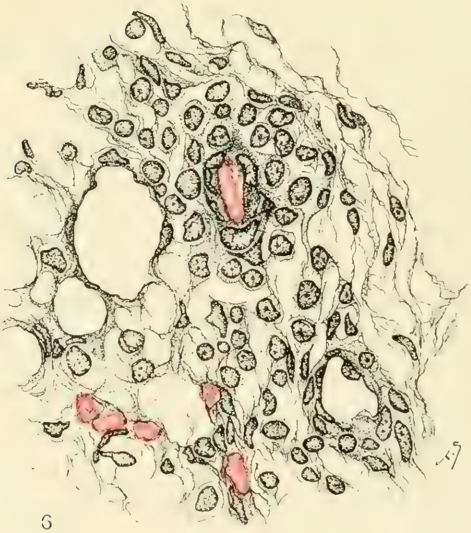

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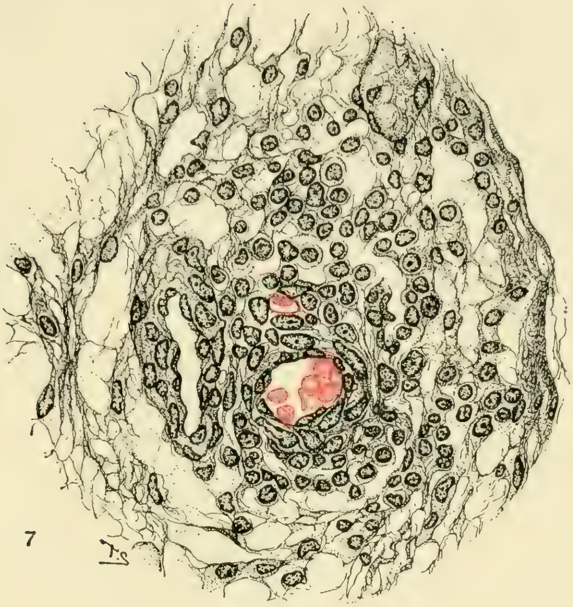
Fig. 5 Marked accumulation of lymphocytes in a developing fat lobule lying very close to a developing hemal node. Foetus 22 cm. × 275.



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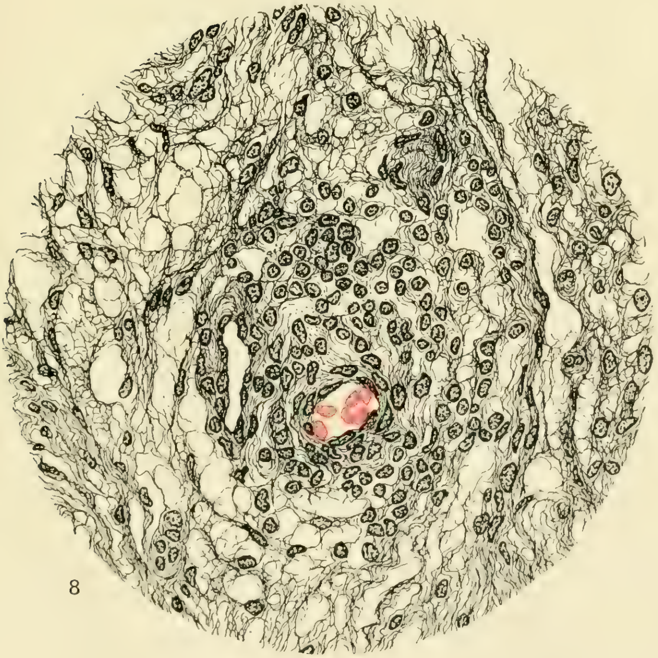

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Figs. 6, 7, and 8 Peri-vascular accumulations of lymphocytes. Foetus 32 cm. × 920, 950 and 345.

ferentiation is but slight. Some cells nearer the center can be distinctly seen, however. Their outline is polygonal and the protoplasm forms a comparatively narrow band around the large circular or vesicular nuclei some of which contain rather large chromatin granules. Slight condensation is present near the periphery and here the nuclei are somewhat more elongated.

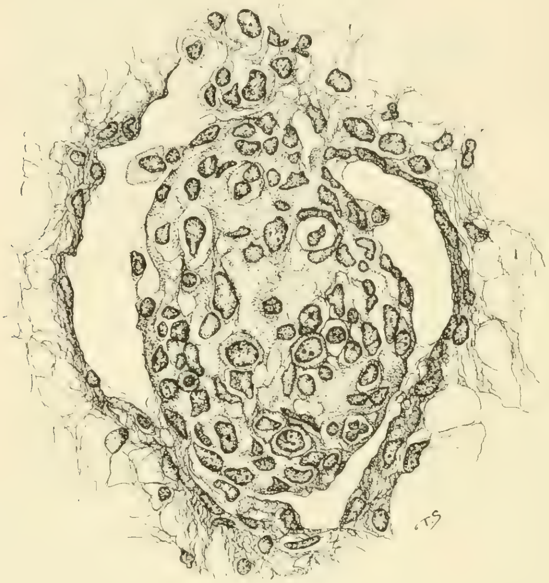

Fig. 9 Very early anlage of a subcutaneous hemal node. Foetus 18.5 cm. × 920.

Although the anlage shown in figure 10 which is about 70 μ largely only shows beginning rarefaction of the peri-nodal mesenchyme the condensation and differentiation within the nodule nevertheless are somewhat farther advanced than in the previous specimen. This nodule too is wholly non-vascular and seems to be entirely unrelated to vessels. It too was found in the subcutaneous tissues and does not lie far from a considerably larger hemal node. In spite of the absence of a definite peri-nodal space the intra-nodal condensation and differentiation has proceeded somewhat farther. No definite cell outlines can be seen but the appearance of the specimen is more uniform, the nuclei are considerably smaller and the beginning of reticulum formation is suggested by the appearance of some of the mesenchyme cells. In some small portions of the periphery the formation of spaces is also indicated.

Although it is difficult to determine the exact relation of the capillaries present in the surrounding mesenchyme to these small nodules neither of them was formed in or near a plexus of any kind. Hence if as I have good reason to believe, these nodules represent very early stages in the development of hemal nodes there can no longer be any doubt as to their formation as independent differentiations in the subcutaneous mesenchyme.

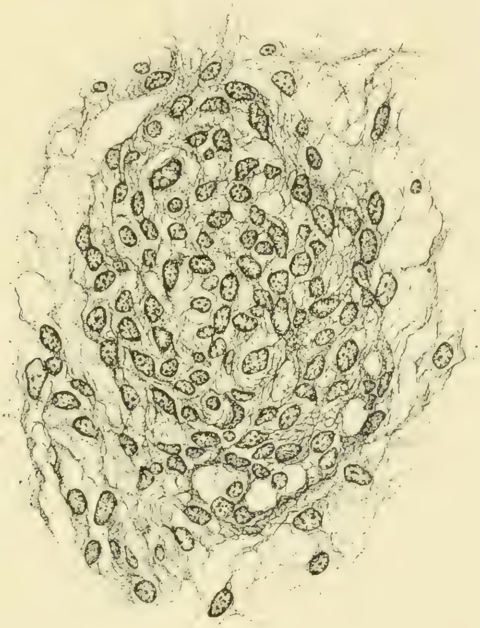

Fig. 10 Somewhat older anlage of a similar node. Foetus 32 cm. × 720.

In the next earliest stage which I happened upon the differentiation of the node has progressed a good deal as shown in figure 11. In this specimen which measures one third millimeter, the syncytium is gone and the cells are quite distinct.

Capillaries and arterioles are found within and near the node and the peripheral sinus is completely filled with erythrocytes and lymphocytes. In spite of the apparently marked advance the capsule shows not the least sign of differentiation, however. The intermixture of erythrocytes within the parenchyma of the node is very slight except at the periphery. There is no unusual indication of the formation of erythrocytes within the node itself. Yet this node is approximately one-fifth millimeter in diameter, practically spherical in form and except for the absence of a capsule resembles some very small nodes taken from adult sheep and bovines very closely in structure. Vascular and lymphatic plexuses are again absent but single or branched capillaries can be seen to enter the peri-nodal space. As shown in figure 12 arterioles also pass through this space to pass into

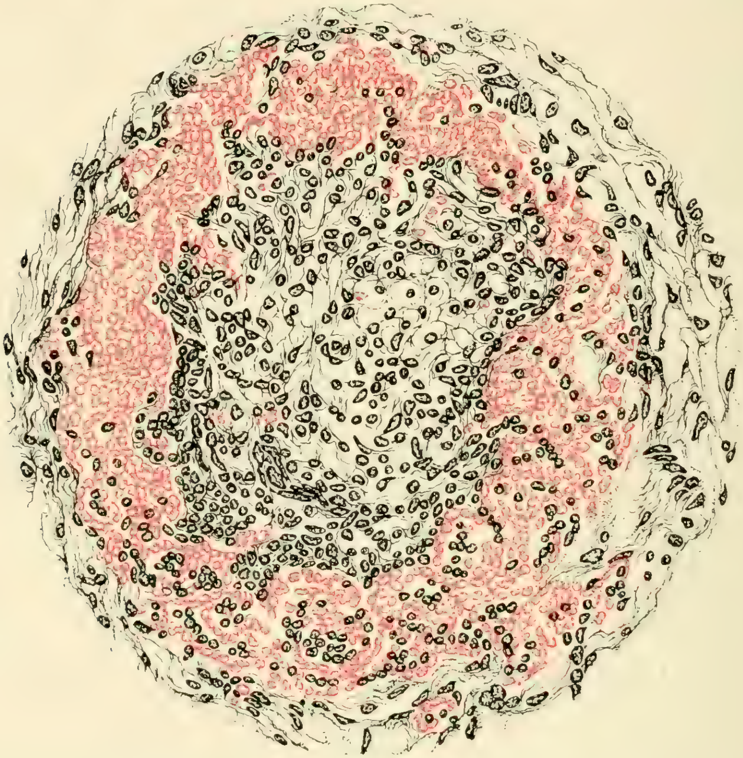

Fig. 11 Considerably older subcutaneous hemal node. Foetus 32 cm. × 350.

the node. The peripheral blood space is decidedly irregular possessing invaginations into the body of the node and evaginations into the surrounding mesenchyme which in sections not infrequently give the impression of being sections of vessels. Few cell outlines are distinctly visible and many of the nuclei appear more like those of older lymphocytes. Some erythrocytes are scattered throughout the node but no intra-nodal sinuses are present. Many of the cells represent a more primitive type however. No lymphatics whatever could be recognized near this node.

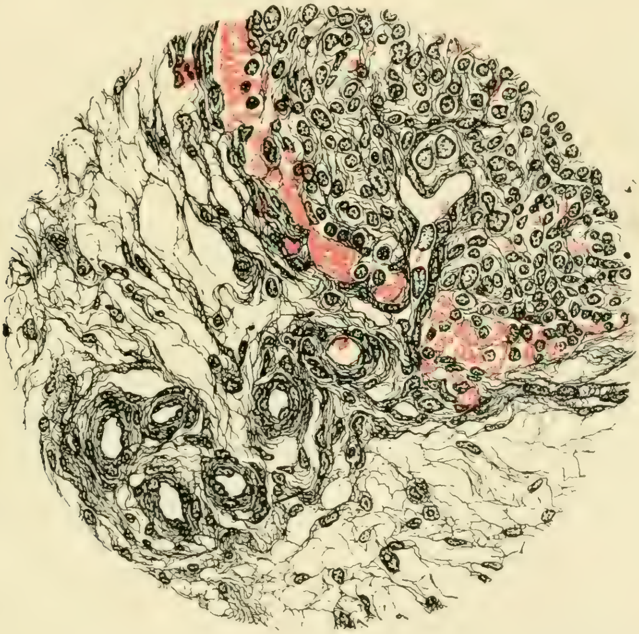

Fig. 12 Arteriole penetrating interior of same node without communicating with the subcapsular blood space. × 475.

A still older stage in the development of hemal nodes is shown in figure 13. In this specimen the capsule still grades directly over into the surrounding connective tissue but the increase in erythrocytes is very marked, especially within the parenchyma of the nodes where they mingle far more freely with the lymphocytes than the drawing shows. Larger arterioles are present and communicate with the periphery and interior but plexus formation is absent in or near the node. The cellular differentiation has greatly advanced but germinal centers are still absent and there is as yet no indication of the formation of the venous lacunae which are so common in nodes from mature animals.

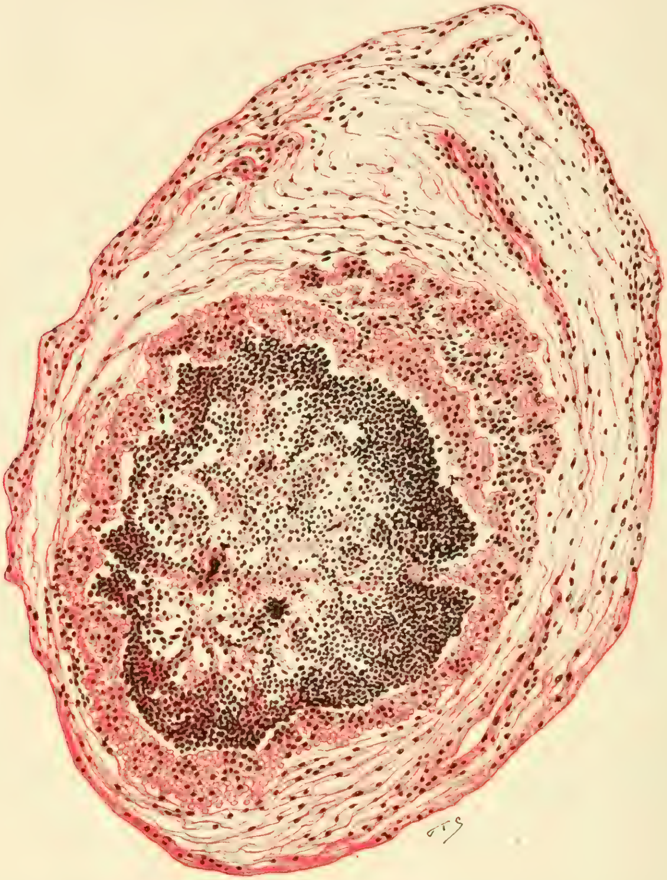

Fig. 13 Subcutaneous hemal node from foetus of 30 cm. × 97.

Vascular and lymphatic plexuses are again absent in this node which is half a millimeter in diameter. It was removed by naked eye inspection from a foetus of 30 cm. The typical pycnotic lymphocyte is the prevailing leucocyte in this node and the advent of giant cells is seen. The lymphocytes are accumulated at the periphery and mingle freely with the blood in the peripheral blood space which seems to possess no definite boundary.

Older stages than those here represented are easily obtainable. Some of these show complete or almost complete development on one end and but slightly differentiated mesenchyme at the other. They suggest that cavernisation and capsule formation may be limited to but a portion of a node for a considerable period of time. The fact that the subcapsular spaces are frequently completely empty of erythrocytes also indicates that the vascular conditions within the node but not necessarily the vascular relations of the latter, vary quite markedly in the early developmental stages as well as later on.

The earliest vascular relations apparently come about through the advent of capillaries which reach the node from the adjacent tissues. The presence of erythrocytes only within the subcapsular space in some nodes, implies no doubt that capillaries may come into relation with this space first. Such a relation is shown in figure 14, taken from the same node. Here the vascular relations are beyond doubt although I am convinced that this direct relationship between the subcapsular blood space and the vascular circulation—except rarely through small confluent veins as reported elsewhere and in wholly depleted nodes—does not exist as a rule in the mature node.

Some of these foetal nodes lay partly imbedded in the underlying musculature but a definite capsule and sometime also the fascia, intervened. Not infrequently hemal nodes which were included in the block of tissue removed from the region of the flank lay near lymph nodes but never in them. In serial sections the presence of a lymphatic plexus or of lymphatic connections of the lymph nodes could usually be easily ascertained, however. Besides the lymph nodes were generally much larger; especially the main ones. This is undoubtedly due to the fact that they develop much earlier, long before the subcutaneous fat lobules. Some hemal nodes develop much earlier, however, and others undoubtedly later. But in spite of these facts and the misleading appearances recorded above, the formation of the fat lobule follows a quite different course than that followed by the hemal node. Hence confusion is impossible except perhaps in its very early forms when the fat lobule too is represented merely by a slight condensation in the mesenchyme as represented in figure 3.

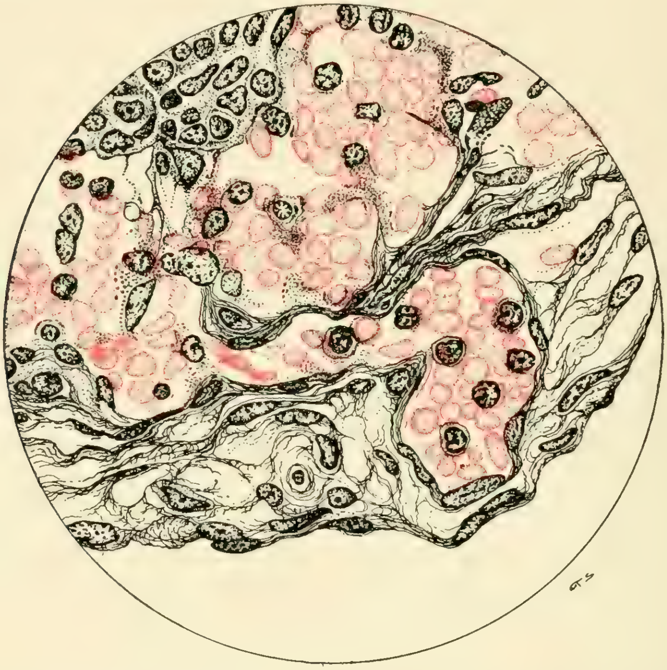

Fig. 14 Peripheral vessel communicating with the subcapsular blood space. Foetus 30 cm. × 1050.

From the evidence obtained so far I feel justified in concluding that the development of subcutaneous hemal nodes in bovines begins as differentiations in the mesenchyme. The earliest anlage is syncytial and the first cells which are circular or polygonal in outline are rather large but are not abundantly provided with protoplasm. The nuclei are large and vesicular the whole cells recalling the large leucocytes of mature lymph nodes. The so-called peripheral sinus seems to form in loco wholly independent of a plexus of vessels but may come into direct relation to the vascular circulation. Independent capillaries and not rarely a group of branching capillaries may come into relation with the peri-nodal space as shown in figure 15 even before the capsule has differentiated or during its differentiation. But the peri-nodal space frequently is incomplete or may never form, and the intra-nodal vessels frequently enter that portion of the node which is still fully undifferentiated. The intranodal circulation is formed by an artery and vein which enter the node by traversing the peri-nodal space which does not as a rule communicate directly with them, a fact abundantly observed in mature nodes of the sheep by Helly, Piltz and myself.

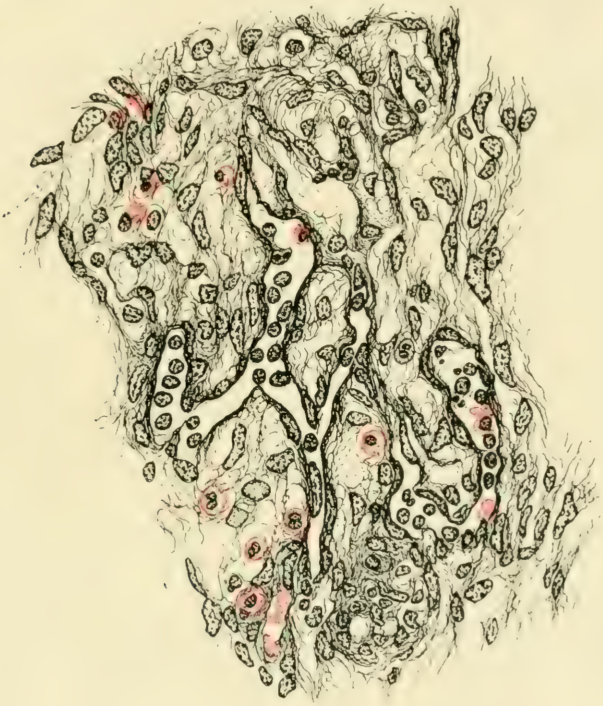

Fig. 15 External section of a developing hemal node showing the relation of the extra-nodal vessels to the subcapsular blood space in the node shown in figure 12. × 475.

Although lymph vessels can be observed in the vicinity of developing hemal nodes I have never seen them come into relation to them nor have I ever seen a hemal node form in or about a lymphatic or vascular plexus. The hemal node then from its beginning is intercalated in the vascular not the lymphatic system.

Clarkson working on the horse, sheep and pig and some of the earliest observers and very recently also Meek '10 working on the pig thought that hemal nodes were the seat of formation of erythrocytes as well as of leucocytes. Robertson '90, e.g., strangely enough, thought that the nuclei of cells which looked like large multi-nucleated leucocytes, became erythrocytes while Meek '10 writing about conditions found in the hemal nodes of the pig says "Of special interest are certain small islets of cells which occur in the midst of the blood in the sinuses. They are sharply defined from the surrounding red blood corpuscles, and are formed from aggregations of various types of blood cells. One such focus will contain, prehaps, fifty closely-packed normoblasts, another a clump of myelocytes, neutrophylic in granulation while yet others are made up of aggregations of polymorphonuclear cells. Mitotic figures may be seen in the cells of these islets. . . . . . The above appearances would seem to suggest that a part at least of the function of these glands in the pig is concerned with the formation of blood cells, red and white and they are described at some length as they differ materially from the conditions found in any human hemolymph glands examined." These observations of Meek are unique, for as emphasized by Drummond '00, the stages in the formation of erythrocytes had not been observed in developing nodes even. However, Drummond too believed that hemal nodes exercised some function in connection with erythrocyte and suggested that this function might by a cyclical one. This conclusion of Drummond's was based on the great variability in cellular content. These variations within the same or in different species, he believed to be due to varying rates of blood-cell destruction. Drummond further thought that the rate of destruction of erythrocytes within the individual node or species, was independent of the number of such nodes present. Warthin '01 who held that the formation of erythrocytes undoubtedly occurred in disease also suggested a cyclical activity and added that "the appearance (in disease in man) often suggests a transformation of adipose tissue into lymphoid tissue and the possibility of a physiological rotation of the two forms of tissue."

Gutig '07 found germinal centers composed of normoblasts and myelocytes rarely present in hemolymph nodes of the pig and hence concluded that the assertion that hemolymph nodes serve only as seats of blood destruction can not be accepted.

v. Schumacher '12 on the other hand, thought it possible that individual erythrocytes are formed in the hemolymph nodes of the sheep but regarded such an origin of erythrocytes as a very subsidiary one. v. Schumacher also reported phagocytosis by reticulum cells and a fragmentation of erythrocytes within phagocytes in some hemolymph nodes but the occurrence of extracellular fragmentation or degeneration of erythrocytes as observed by Weidenreich is denied. v. Schumacher like myself also observed but few pigment cells.

That the destruction of erythrocytes both by extra- and intracellular disintegration and hemolysis and the formation of leucocytes occur within hemal nodes is undoubted. That these processes vary extraordinarily in degree within different nodes of the same individual species, as well as in those of different species is also evident. I fully realize that it is rather venturesome and often quite futile to draw conclusions regarding function from a purely morphological basis, yet it seems to me that the supposed function of 'blood destruction' has been wholly over-emphasized. Moreover, it might pertinently be suggested that it is after all not 'blood destruction' but mainly destruction of erythrocytes which is apparently so preëminently a characteristic of some hemal nodes. Nevertheless, since so many hemal nodes contain so very little blood even when practically depleted of lymphatic tissue, it is highly improbable that destruction of erythrocytes is the chief or even as important a function of hemal nodes as would seem to be the case upon cursory examination. For even if destruction of erythrocytes, be it by phagocytosis or by erythrolysis associated or unassociated by cytorrhexis, is the chief function of hemal nodes, it is evident, of course, that these processes must in all probability be due and largely if not solely due, to some activity—direct or indirect—on part of the parenchyma of the hemal node. Hence, even if this destruction within the node were due in part to the production of some lysin or enzyme, one might reasonably expect the quantity of lymphatic tissue to be considerable or proliferation of lymphocytes most active, in nodes containing comparatively large quantities of blood. This is, however, not the case for as is evident, the quantity of lymphatic tissue must of necessity vary inversely with the quantity of contained blood and it is only rarely that many follicles are found in a depleted node or, for that matter, in any hemal node which contains much blood. But even when this is the case and rapid proliferation of lymphocytes occurs, this very activity would then defeat the main object for which these nodes are supposed to exist—viz., blood destruction. For just in proportion as the hemal nodes became more able to destroy blood, less of the latter would be able to stay in the node since the more lymphatic tissue there is, the less blood can be accommodated in the parenchyma of the node. It is, of course, mainly if not wholly in the latter and not in the vascular current within the node, that the destruction of erythrocytes is believed to occur. Then too, it is probable that the volume of flow through the node necessarily increases, up to a certain point at least, when the node is undergoing depletion as a result of loss of lymphocytes by the blood stream, and undoubtedly also as a consequence of the exercise of the supposedly specific functions of the node. Consequently, the more rapid the flow and the larger the quantity of blood that can be accomodated within the node the smaller the power of the node will be to perform the very task for which is is supposed to exist. That is, just in proportion as the need for the exercise of their function of blood destruction increased the nodes necessarily would become progressively less competent to fulfill it. Hence rapid self-destruction would seem to be the inevitable consequence of the exercise of a supposedly normal physiological activity. To be sure, this is the ultimate fate of every organ and organism, but it seems unlikely that organs which apparently function for comparatively long periods of time should be subjected to such rapid self-destruction at the very time when according to this assumption, the need for their activity becomes greater. Since, moreover, the quantity of blood in some hemal nodes, is so very insignificant and since it varies so exceedingly in quantity and particularly since no signs whatever of destruction of erythrocytes can be noticed in many nodes, it is difficult indeed to regard such a function as the only or even the chief rôle which hemal nodes play in the economy of the organism. Hence, for this and also for other important considerations, the designation 'hemolytic organs' suggested by Warthin does not seem wholly justified. In view of the above facts it seems more probable to me that the formation of leucocytes of various types rather than the destruction of erythrocytes is the chief function of the hemal nodes.

That eosinophiles are formed in hemal nodes one can scarcely doubt. At least if they are not formed there in the sense that all of them are cells which are newly formed within the node itself, there seems to be no escape from the conclusion that eosinophile granules form within these nodes, in large numbers of cells which previously contained none. Moreover, phagocytes are also undoubtedly formed there. The largest of the latter, the polykaryocytes and some megakaryocytes could, to be sure, not easily leave the node or be transported there because of their great size and must hence have arisen within the nodes themselves or have been transformed there. Since these poly- and megakaryocytes are relatively few, their activity whatever it may be, is probably not a very important or, at least, not a very pronounced one. Most of them look indeed like dying cells.

The supposition that formative or conservative rather than destructive processes prevail in hemal nodes receives some support from the observations of Warthin and others to the effect that the hemal nodes of man—if such there be—are enlarged in diseases in which blood destruction is known to occur. Moreover, no one has regarded the cause of blood destruction in these diseases to lie in the enlargement of these nodes, and it would seem more probable that hyperactivity in them at such a time —as a result of infection or the production of toxine—should as in the case of the bone marrow, be regarded as a constructive rather than as a primarily destructive reaction or process. Then, of course as has been suggested repeatedly, there is the possibility that hemal nodes have the same function as the spleen and supernumerary spleens—whatever that may be.

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THE POSITION OF THE RESPIRATORY VASCULAR NET IN THE ALLANTOIS OF THE CHICK

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ONE PLATE

The structure and function of some of our organs seems to be so well established, that it requires a certain courage to communicate new observations in fields which seemed to be exhaustively studied. My observations refer to the arrangement of the respiratory capillary net in the allantois of the chick.

In the chick embryo from the sixth day of incubation until hatching, the capillaries of the allantois subserve the function of respiration. The allantois is a sac, the walls of which are flattened by the narrow space offered to its development and can be considered as two sheets. The outer sheet contains the richly developed respiratory net, which, according to Füllborn's[1] expression, can be compared with a sinus traversed by trabeculae of mesodermal tissue.

The respiratory capillaries of the outer sheet of the allantois are homologous in their function, according to Schulte's terminology homodynamous, to the respiratory capillaries in the placenta of the mammalian embryos and to those of the lungs and the gills in the definitive respiratory organs of vertebrates. The respiratory capillary net manifests in its structure as well as in its relation to the constituent parts of the organs, in which they develop, perfectly similar features. No where seem the respiratory capillaries to come into immediate contact with the air or with the fluid, which contains the oxygen. The endothelial wall of the capillaries is separated from the immediate action of the air or the liquid by a special membrane, which consists of ecto- or entodermal cells.

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[1] Fried. Füllborn, Beiträge zur Entwicklung der Allantois der Vögel. Inaugural Dissertation. Berlin, 1895.

This situation of the respiratory capillary net is merely a special case of the general arrangement of the mesodermal structures in the organisms and necessarily ensues from the primitive development of the *mesoderm between* the two other germlayers. To state that the definite localization of this tissue becomes in itself a differentiating factor would seem to repeat a commonplace. A mesodermal cell acquired definite potencies, and has lost certain others in association with its development not on the surface but between the surfaces.

Whether the definite localization between two germlayers in the course of development has become for a mesodermal cell a necessary condition for its existence has hitherto seemed subject to little doubt. The fact that both in embryos and in the adult organism, mesodermal structures especially those of mesenchymal type are separated from the outer environment by ecto- or entodermal membranes is well established through all the animal classes. There still exists a curious exception from the usual relations between the derivatives of the germlayers, which is seen in the arrangement of the respiratory capillary net in the allantois of the chick. Though the definite localization of the mesodermal tissues is their most important character at their appearance, it does not necessarily follow, that it invariably remains one of their characteristic features. Mesodermal structures which have appeared between the two primitive germlayers might grow through one of them and develop here further into specific structures. Though instances of such relations between the derivatives of the germlayers have not yet been described, an example is furnished by the capillary net of the chick allantois.

This membrane has been recently studied by Füllborn.[2] I am not, therefore, endeavoring to give in my short note a full account of the development of the allantois itself or of its capillary net. The study of a series of allantois between the sixth and twenty-first day of incubation, gave, however, additional data concerning the relations of the capillary net to the ectodermal layer of the allantois, which previously belonged to the serosa proper. At the beginning of their development all the vessels are subepithelial; soon, however, a rich capillary net grows and develops inside of the epithelial layer itself, pierces the epithelium and expands freely above the surface of the ectoderm immediately under the egg shell membrane.

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[2] Loc. cit.

In my previous paper on the equivalence of the hematopoietic anlages,[3] I had the opportunity of mentioning the extreme vulnerability of the vessels of the allantois in later stages. This vulnerability and the ensuing hemorrhages are now fully explained by the development of the respiratory capillary net above the ectoderm under the egg shell membrane.

This peculiar localization of the respiratory capillary net in the outer sheet of the allantois did not entirely escape the observation of Füllborn.

> Dort wo die Kapillaren des äusseren Blattes ihre typische Ausbildung erlangt haben, finden wir statt des doppelschichtigen kubischen Serosa-Epithels eine Schicht sehr platten Zellen, welche unmittelbar über den Kapillaren liegen; es erinnert dies ganz an die Verhältnisse, welche wir sonst bei respirierenden Organen zu finden gewohnt sind. Diese dünne Ektodermlage wird in dem Laufe der Entwicklung von den ihr anliegenden Wandungen der Kapillaren immer schwerer zu unterscheiden und in der zweiten Hälfte der embryonalen Entwicklung gelingt dies meist nicht mehr.

This statement quoted from Füllborn's paper[4] strongly suggests an immediate contact between the respiratory capillary net and the egg shell membrane, but according to Füllborn, it seems to have resulted from a secondary degenerative process to which the ectodermal cells succumb. The stage of intra ectodermal localization of the capillary net evidently was not observed by Füllborn. An epithelial-like layer of cubic cells beneath the capillaries is, however, mentioned, but they were interpreted as of mesodermic origin. Thus the outer sheet of the allantois in Füllborn's description seems to be deprived of ectoderm, and so again reduced to its primitive two layers of entoderm and mesoderm.

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[3] Equivalence of different hematopoietic anlages 1. Spleen. The Amer. Jour. of Anatomy, vol. 20, 1916, p. 255.

[4] Loc. cit.

The two figures 1 and 2 illustrate the process of the development of the capillary net on the surface of the allantois. At no stage does the epithelial ectodermal membrane degenerate; it is invariably present, but it changes the superficial position which it occupied in earlier stages and is found in later stages below the capillary net. There is little doubt, that the epithelial layer of cubic cells observed by Füllborn and thought to be of mesodermal origin is the ectodermal layer of the serosa missing in his description.

Figure 1 represents the superficial layers of the outer sheet of the allantois. The ectodermal membrane is at this stage well defined; its constituent cells are easily identified; their large light nuclei with a well developed nucleolus and a net of chromatic filaments markedly differ from those of the mesodermic cells; their cytoplasm, stained with Eosin Azur, takes a purplish tint, while the mesodermic elements show a blue-greenish color. No definite boundaries are seen between the cells of the ectodermal layer, and their respective limits are merely indicated by regions of somewhat acidophylic cytoplasm. The more superficially situated ectodermal cells appear flattened. Numerous mitoses are encountered.

Figure 1 represents only the superficial parts of the outer layer of the allantois. A branch of a deeper situated vessel runs perpendicularly to the ectoderm membrane, penetrates it and here resolves itself into numerous meshes of a capillary net. The meshes are situated in the substance of the ectodermal membrane and are intimately surrounded by ectodermal cells. They have their own walls which consist of a thin endothelium with numerous nuclei. These nuclei are small and flattened, possess numerous small chromatic particles but no visible nucleoli and take a marked greenish tint. The meshes of the net situated in the ectodermal layer are derived from the vessels which grow into and pierce the ectoderm. They are filled by circulating blood in greatest part by erythrocytes, though granular leucocytes and occasionally hemoblasts are seen.

A peculiar interaction between ectoderm and ingrowing vessels can be observed during the earlier stages. The ectoderm cells of the deeper layers in the vicinity of the vessels undergo a rearrangement and form conical projections directed toward the vessels; individual ectodermal cells send out processes toward the vessels, as if they were actively attracted by them. Intimate connections are established between the ectoderm and endothelial cells and the two structures would be indistinguishable from one another were it not that the large nuclei of the ectodermal cells and the specific staining reaction of their cytoplasm offered criteria adequate to differentiate them from the mesodermic elements of the vascular walls.

In somewhat later stages the intraectodermal position of the respiratory capillary net undergoes a further modification. At the thirteenth to fifteenth day of incubation the capillary net is situated above the ectoderm. The meshes, in earlier stages surrounded by epithelial ectodermal cells, now come at least by one of their surfaces into immediate contact with the egg shell membrane. The net itself becomes gradually closer, the individual meshes touch each other and in a few places only are separated by small islands of ectodermal tissue. The lumina of the capillaries are surrounded by a distinct endothelium, the nuclei of which markedly differ from the nuclei of the ectodermal cells. The capillary net now situated immediately under the egg shell membrane is connected by numerous anastomoses with the larger vessels of the deeper mesodermal layers of the allantois.

At this stage the ectodermal epithelial membrane is found entirely beneath the capillary net, it is traversed by the numerous anastomoses between larger vessels and the capillary net; which lies on its surface. The ectodermal cells retain the characteristic features of the earlier stages. Only a few of them reach the surface of the allantois projecting between the individual meshes of the net and come into contact with the egg shell membrane. The structure of the outer sheet of the allantois offers an example of unique relation between the derivatives of the germlayers. The derivative of the mesoderm, the capillary net, actually traverses the ectodermal membrane and expands above in the form of a rich plexus. First developed between the germlayers the vessels are not necessarily confined to this location and seem to be perfectly adapted to an existence within the ectoderm as well as on its free surface.

Localization of vessels inside of the epithelial membrane is not wholly unknown.[5] Even within the trophospongium of a cell capillaries have been observed.[6] An expansion of a capillary, particularly of a respiratory net above the surface of the epithelium, so far as I am aware, has not been observed. Of course, even in this case, the vessels are not in immediate contact with the air which contains the oxygen; they are covered by the egg shell membrane and by the egg shell, but neither of them are living cellular layers, and the oxygen reaches the endothelial wall of the capillaries without passing through a living substance.

A study of the gradual development of the allantois has lead me to a different interpretation of the subvascular epithelial-like cells from that given by Füllborn. This layer of cubic cells, found beneath the capillary net is according to him of mesodermal origin, but in reality they are the ectoderm of the serosa. Recent studies tend to show that the morphological structure of the individual cells does not present criteria adequate for their identification, and this is especially true for young undifferentiated cells. Even less information concerning the origin of the tissue can be sought from the study of the specific arrangement of cells in tissues; epithelial membranes are formed by ecto- and entoderm as well as by mesoderm; reticular tissue may derive from any germlayer. Inasmuch as the structure of the nuclei and of the cytoplasm of the epithelial cubic cells beneath the vascular net markedly differ from the cells of the endothelial capillary walls, it is permissible to speak of two different structures. The ectodermal origin of the epithelial membrane cannot be however necessarily deduced either from its specific structural features or from the epithelial arrangement of its constituent cells. The localization of the ebithelial like cells in this particular case might however offer more or less evidence of their ectodermal origin. Epithelial-like membranes, disregarding their origin, are usually formed on free surfaces or around lumina, and the existence of an epithelial-like membrane in the midst of mesodermal structures must strongly suggest its secondary development at the expense of a structure, formerly otherwise situated.

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[5] For references see: Traité d'histologie. A. Prenant, P. Bouin et L. Maillard, p. 86.

[6] Textbook of the principles of animal histology. U. Dahlgren and W. A. Kepner, p. 7.

The identification of the subvascular epithelial-like cells as ectodermal can be established by the study of their histogenesis, but this method must be applied exhaustively. A gap in observation leads often to a wrong conclusion, and the lack of stages, which showed a growth of the capillary net through the ectoderm led Füllborn to the conclusion of the mesodermal origin of the subvascular epithelial-like cells.

A reliable method of identification of cells and tissues can be found in the study of their potencies for differentiation. If two kinds of differently constituted seeds cannot be distinguished, let them grow under equal environmental conditions; their products of development will be different, if the seeds were. In a previous paper[7] concerning the small cortical thymic cells, the latter were identified as true lymphoid elements on the basis of this method. This conclusion was drawn from the experimental result that the stemcells of the small cortical thymic cells under definite conditions differentiated into granular leucocytes. The structural characters of the cortical thymic cells and those of their stemcells long seemed insufficient for their final identification. The recourse to a study of the potencies of the stemcells in the thymus served to establish definitely their identity as true hemoblasts; hence the small cortical thymic cells, as derivatives of hemoblasts, are not disguised epithelial cells of entodermal origin, but true small lymphocytes.

The same method can be applied to the identification of the subvascular epithelial-like cells in the allantois. The recognition amongst the derivatives of these cells of differentiation products exclusively characteristic of a given germlayer, would be adequate proof of the derivation of the cells in question from that germlayer. If by means of experimental intervention the cells of the subvascular epithelial layer were transformed into hemoblasts, their mesodermal origin would be definitely proved. If, on the contrary, a cornification of this layer could be obtained, this would offer strong evidence for their ectodermal nature.

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[7] The differentiation of cells as a criterion for cell identification, considered in relation to the small cortical cells of the thymus. The Jour. of Experimental Medicine, 1916, vol. 24, p. 87.

The allantois was used in my recent studies[8] on the potencies of the reticular splenic cells as a favorable medium for growth of tissues and development and differentiation of their cells. The various tissues of the allantois, under the condition of the experiments, manifested definite changes; most of them were of progressive nature. Those relating to the subvascular epithelial layer only are of value in connection with the identification of its constituent cells.

The changes in the cells of the subvascular layer were revealed in response to the irritation produced by the apposition of different tissues on the surface of the allantois. Though in nature similar, they differed somewhat according to the stage at which the cells were activated in the course of the experiment.

A proliferation was brought about not only in cells which were in immediate contact with the graft but also in regions at a distance of 2 to 5 cm. from the graft. A proliferation took place not only at stages in which the epithelial-like layer covered the allantois as a membrane but also at stages in which it occupied a deeper position beneath the vascular respiratory net. In both cases the ultimate changes undergone by the epithelial layer of cubic cells consisted in cornification of the more mature cells.

In stages, in which the cubic cells formed a membrane on the surface of the allantois, their proliferation led to a thickening of the membrane. As seen in figure 3 the epithelial membrane of the allantois is now composed of numerous layers of epithelial cells, amongst which in many places three or four different kinds of cells can be recognized. The whole structure of the membrane now reminds one strongly of the squamous epithelium of the epidermis. The deepest group of cells are cylindrical or cubic, basophylic and numerous mitoses are found amongst them. Layers of more flattened and less basophylic cells separate the younger cells from a stratum which shows definite and characteristic changes. Numerous particles of kerato-hyaline appear in the cytoplasm of these cells and transform a whole layer of the epithelial membrane into a stratum granulosum. The outer layers of the membrane undergo in many places a complete cornification. Of course the process has not the regularity seen in the epidermis; for example, numerous vacuoles develop especially in cells on the surface of the membrane, as seen in figure 3, but the cornification is sufficiently well pronounced to leave no doubt of its significance: the cells of the epithelial-like layer may undergo a cornification; this is a change exclusively characteristic of ectodermal and entodermal derivatives, therefore they must be considered as being of ectodermal and not of mesodermal origin (entodermal origin being out of question).

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[8] Potentialities of the lymphoid hemoblasts of the adult spleen. Proc. of the Am. Assoc. of Anat., 1917, p. 345.

Interesting changes are observed in the epithelial cubic cells if they are activated at a time when a vascular net has developed above it or in its substance. In the latter case the intensive proliferation of the epithelial cells leads to a discontinuity of individual meshes of the capillary net; the blood corpuscles are then ingested by endothelial cells. Cells of the epithelial membrane readily arrange themselves around small accumulations of blood corpuscles or endothelial cells, which soon become necrotic and the whole structure is very similar to an epithelial pearl or to a Hassal's corpuscle. Even more characteristic pictures arise from the proliferation of the cubic cells when they occupy a subvascular position. Solid sprouts of ectodermal tissue often develop from the deeper layer of the epithelial-like membrane, they push into the mesenchyme of the allantois and here form well defined agglomerations of epithelial tissue. Never do the cells of this tissue separate from one another, the tissue may be traversed by numerous ameboid cells, which finally may transform it into a kind of reticulum, like the entodermal tissue in the thymus, but within the allantois they do not break up into individual cells and invariably retain their reciprocal connections. Groups of epithelial cells are however found separated from the larger accumulations and here often develop into typical pearls.

The cornification of the embryonic cubic cells after a activation which transformed the thin subvascular layer into a stratified epithelium may serve as a conclusive proof of their ectodermal origin. The superficial localization of the respiratory capillary net is therefore not the passive result of a supposed degeneration of the ectodermal cells above it, but it is due to an active growth of the vessels into and through the ectoderm. The latter is found secondarily lying beneath the vascular net normally in the form of a double layer of cubic cells.

The study of the allantois has shown an example of a peculiar rearrangement of the derivatives of the germlayers; tissues of mesodermic origin appear on the surface of the allantois and an ectodermal epithelial membrane is thereby pushed deeper into the mesenchymal layer of the allantois. Both structures retain in their new position their specific characters. Moreover the ectodermal membrane, the cells of which continue to proliferate under the new environment given by experiment, develop and differentiate in a definite manner elsewhere exclusively characteristic of epithelial ectodermal tissue. This may suggest, that the ectoderm of the serosa has reached a stage at which the cells have acquired a specificity sufficient to make them differentiate along definite lines under any environmental conditions.

PLATE

PLATE 1

EXPLANATION OF FIGURES

The figures were drawn with the camera lucida at stage level with Zeiss apochromat 2 mm. oil immersion obj. and the compensatory ocular 6.

The figures represent outer parts of the external sheet of the allantois.

1 Normal allantois at the tenth day of incubation.

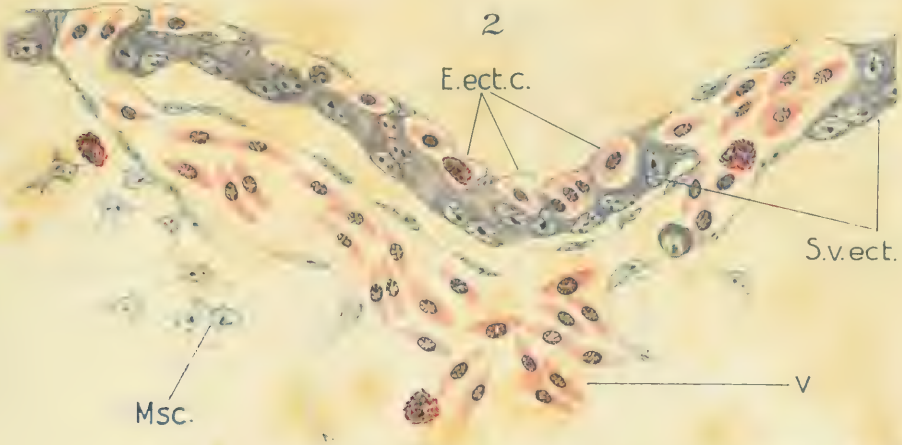
2 Normal allantois at the fifteenth day of incubation.

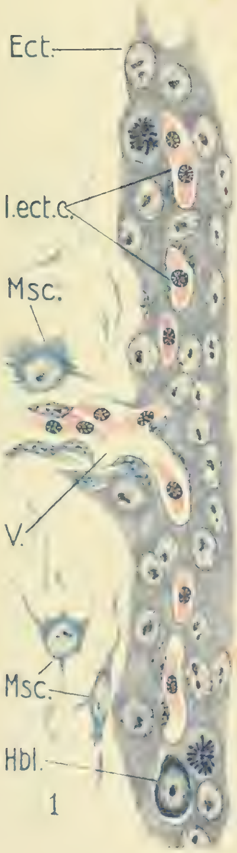
3 Cornification of the ectodermal layer in the allantois at the fifteenth day of incubation around the graft made at the eighth day of incubation.

ABBREVIATIONS

Ect., ectoderm of the serosa
E.ect.c., epiectodermal capillary net
Hbl., hemoblast
I.ect.c., intraectodermal capillary net
Msc., mesenchyme
S.v.ect., subvascular ectoderm
v., vessel.

2





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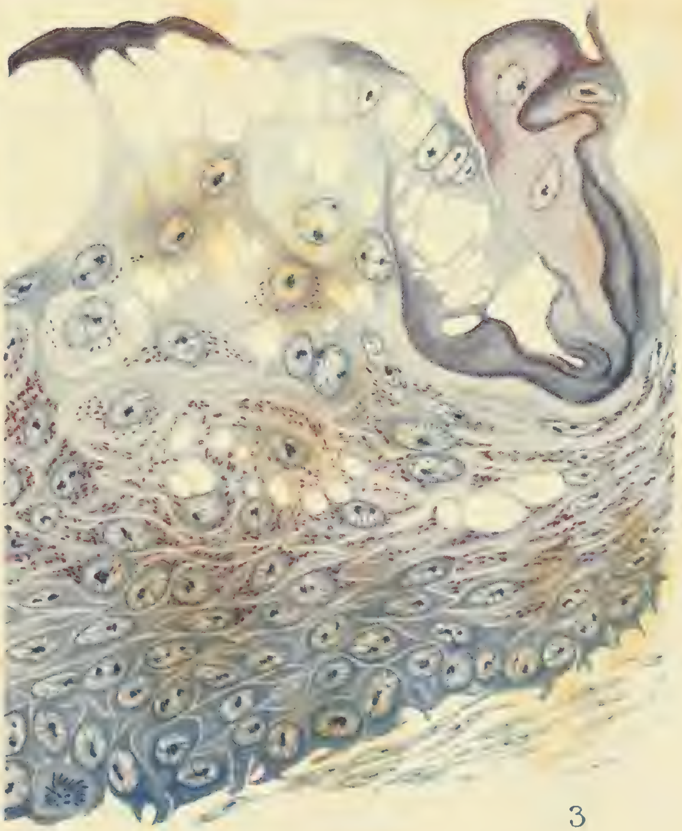


3

A STUDY OF THE REACTION OF LYMPHATIC ENDOTHELIUM AND OF LEUCOCYTES, IN THE TADPOLE'S TAIL, TOWARD INJECTED FAT

ELIOT R. CLARK AND ELEANOR LINTON CLARK

From the Anatomical Laboratory of the University of Missouri

NINE FIGURES

INTRODUCTION

The present investigation is part of a series undertaken in order to study the growth and reactive powers of living tissues and cells in the tadpole's tail. These experiments were planned particularly in order to find out whether lymphatics react, by growth processes, to the stimulus of specific external substances, with the ultimate object of determining, if possible, what regulates the growth of lymphatic endothelium. Such a study involves observations on other tissues, such as blood vessel endothelium, mesenchyme cells, and leucocytes.

The transparent tail of the frog larva is admirably adapted to the experimental investigation of the growth and reaction of these types of cells and tissues because of the readiness with which this animal lends itself to experiment and to observation under chloretone anaesthesia, because of the ease of watching the behavior of each cell, in the living animal, and also because the normal mode of growth of the tissues present in the tadpole's tail has been thoroughly established.

One of the authors (3, 4), in extensive studies in which every capillary and mesenchyme cell, for certain regions of the same tadpole, were carefully watched and recorded, for periods of several weeks, has shown that, at the stage when the tail has become transparent, connective tissue cells, wandering cells, blood vessels, and lymphatic endothelium are each specific and independent tissues. Hence, in studying problems dealing with the growth of lymphatics and blood vessels in the tadpole's tail, the question of the primary differentiation of endothelium from mesenchyme is not involved. But the question of the nature and reactive powers of the lymphatic capillary, its relation to the tissue spaces, and its rôle in the process of absorption can be investigated to advantage in living tadpoles. The importance of some of these problems has been emphasized by Miss Sabin (26) in a recent summary of the investigations on the growth of the lymphatic system.

Our interest in the present problem started with the observation by one of the authors (3), published in 1909, that lymphatic endothelium, in the tail-fin of the tadpole, reacts to the presence in the tissue spaces of red blood cells which had been extruded by pressure from the blood capillaries. In such cases, lymphatics grew out to the blood cells and actively engulfed them. Since the morphological changes which the lymphatic underwent, in reaching blood cells at a distance, were similar to the usual growth changes of lymphatic capillaries, it was suggested that the normal growth of lymphatic endothelium, after its primary differentiation, represents a response to specific substances outside, which are usually invisible.

That specific external substances may be a growth regulating factor for lymphatic endothelium, was also suggested by Evans (8, 9), who found that lymphatics fail to grow into tumors of epithelial origin, while they do invade tumors of connective tissue origin. He proposed the hypothesis that, in the first case, there are present angio-repellant substances, and, in the second case, angio-tactic substances. This hypothesis has not been tested.

It occurred to us that it might be possible, by injecting certain substances into the transparent fin expansion of the tadpole's tail, to gradually obtain information as to whether the lymphatic endothelium is influenced by them and, if so, by what types of substances. The selection of substances which presumably might have a specific attraction for lymphatics was preceded by injections of an inert substance. The results of injecting globules of paraffin oil, a substance whose only action could be mechanical, have been published (5). Small globules of the paraffin oil, injected subcutaneously into the fin, were found to exert no influence on mesenchyme cell, blood vessel or lymphatic endothelium. A transient gathering of leucocytes (sometimes only five or six of these) which always subsided three or four days after the injection, was the only observable tissue response. The globules had not diminished in size after two weeks of observation.

On another occasion, a few granules of India ink were injected into the tail-fin and the result observed under the compound microscope. In this case, the carbon granules were taken up by the processes of the connective tissue cells and by leucocytes, and four or five hours after the injection, most of the granules had been disposed of in this way. This experiment, the details of which will be published elsewhere, showed that the ordinary connective tissue cells, in the tadpole's tail, possess a phagocytic power toward carbon granules which is not shared by blood-vessel or lymphatic endothelium.

For the present experiments, which were carried out in the spring of 1916, we selected fat, in various forms, as a substance which might conceivably exert an attraction on lymphatic endothelium. This was suggested by the presence of an extremely rich lymphatic supply to the intestine of adult vertebrates and of the association of these vessels with fat absorption, and also because certain analyses of lymph, collected from lymphatics draining the leg, have shown a decidedly higher fat content than that of the blood plasma. It was also suggested because of the fact that fat globules can be clearly seen and any diminution in their size easily noted.

MATERIAL AND METHOD

The tadpoles, used for these studies, were the larvae of Rana pipiens. The substances, selected for injection, were olive oil, oleic acid, cream and yolk of egg. The tadpoles were anaesthetized in chloretone (1 to 3000) and a small amount of the substance to be tested was injected subcutaneously into the transparent fin expansion of the tail. The injections were performed under the high power of the binocular microscope, with glass canulae measuring about 20 micra at the tip. Before injection, the fat was heated in a water bath to a temperature of 70°C., but was not boiled in order to avoid an alteration in its chemical constitution. The olive oil and oleic acid were introduced in the form of single globules, measuring about 50 micra in diameter. The cream and yolk of egg, both of which consist mainly of an emulsion of very fine droplets, were injected in the form of a small irregularly shaped mass of approximately the same size as the globules of oil. The various substances were injected in different locations in different larvae; in some cases, they were introduced near the edge of the fin at a distance from lymphatics and blood capillaries, in others, they were injected near the muscle edge and consequently nearer the vessels.

The anaesthetized tadpoles were then observed, in chloretone (1 to 5000) under the compound microscope, in a micro-aquarium, by a method previously described (4). The cells and vessels in the neighborhood of the injection were drawn, and records made of them from day to day.

Description of experiments

The results of injecting each of the substances used will now be described:

1. *Olive oil.* As previously stated, the substance was injected into the subcutaneous tissue of the tail, under the binocular microscope and with the aid of fine glass canulae. The anaesthetized tadpole was then transferred to the micro-aquarium and the oil and neighboring mesenchyme cells, blood-vessels and lymphatics were drawn with the aid of the Leitz drawing eye-piece No. 164. Immediately after injection, the oil globules measured from 30 to 70 micra in diameter and were, as a rule, oval in shape. From ten to twelve hours later they became spherical. On one or two occasions in which the site of injection became infected, the olive oil was extruded from the tail. In

most cases, however, the globule retained approximately the same position throughout the period of observation.

Soon after the injection of olive oil, leucocytes were observed to pass through the walls of nearby blood-vessels and to wander toward the oil. Upon reaching it, they flattened out and formed a ring of cells around the periphery of the globule. A few minutes after coming in contact with the oil, the leucocytes

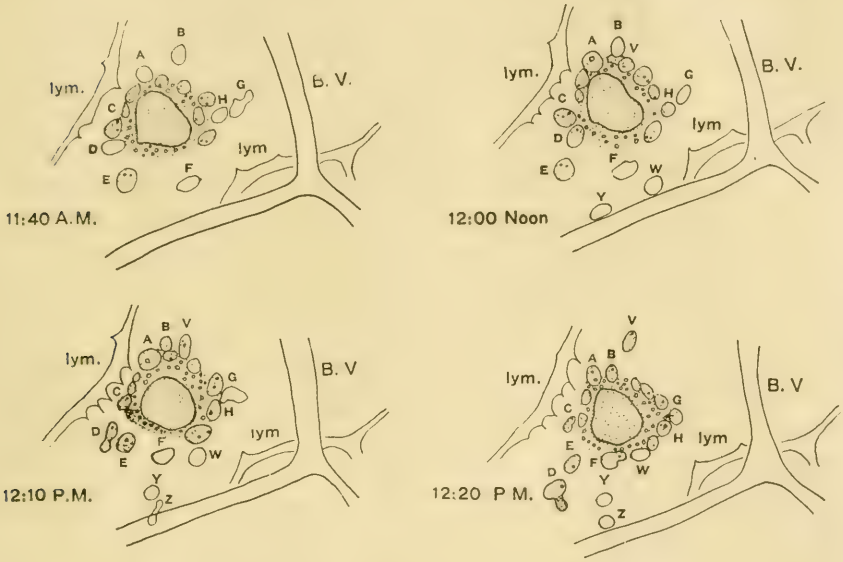


Fig. 1 Series of records illustrating the first reaction of leucocytes toward the various substances injected. In this case a globule of oleic acid was injected eighteen hours before this series of sketches was drawn. Immediately after injection it changed to a brown opaque mass. At the time of making these records it consisted of a dense brown central portion and an outer portion, containing small refractile oil globules and surrounded by a ring of leucocytes. During the observations, leucocytes were seen to pass out of neighboring blood vessels and to approach the injected mass, where they were seen to take up the brown pigment and small oil globules. Some of the leucocytes containing pigment were seen to wander away. These records show clear leucocytes *A B D G H* and *F* approaching the injection mass and becoming pigmented. *W*, *Y* and *Z*, leucocytes which came out of the blood vessels and moved toward the injected mass. *C D* and *V*, pigmented leucocytes which wandered away from the injected mass. *B.V.*, blood vessels; *lym.*, lymphatic. Enlargement = 168x. Drawn with camera lucida.

became pigmented. This pigment consisted of numerous small brown granules, the larger of which looked clear and refractile by transmitted light and were apparently small drops of oil.

When this region was observed and drawn at intervals of a few hours, it was obvious that lymphatic capillaries were attracted by the presence of the oil. A branch from a nearby lymph vessel, soon showed a tendency to grow in the direction of the globule, even bending out of its course to do so, and reached the rim of pigmented leucocytes within a few hours, or after two or three days, depending upon its distance from the globule at the time of injection. After reaching the oil, the tip of the lymphatic remained in close contact with the encircling leucocytes for several days. Occasionally, the tip of the lymph sprout later extended beyond the oil, and in such instances the wall of the lymphatic remained in close proximity to the pigmented leucocytes and to the globule. No pigmented leucocytes were seen to enter a lymphatic.

The exact relationship of the lymphatics to the oil and its rim of leucocytes was often difficult to determine. In other cases, such as the one illustrated in figure 6, the peculiar relationship could be observed to better advantage. In such an instance, a leucocyte containing oil droplets of varying sizes could be seen, closely adherent to the tip or wall of the lymphatic capillary which had grown out to the globule. Often a leucocyte remained in such a position with relation to the lymphatic for as long as six to eight hours and, during this period, the larger oil drops gradually became smaller and finally the very small droplets and granules decreased in amount and the leucocyte became clear. The process was more readily understood after watching a similar occurrence in the case of the other injected substances, where the reaction takes place more rapidly, and will be described in greater detail in that connection.

A number of times during the period of two to three weeks in which the absorption of injected olive oil was observed, small globules about one-tenth to one-fifth the size of the main globule became separated from the rest of the oil. These smaller globules were engulfed by leucocytes, although in many cases they

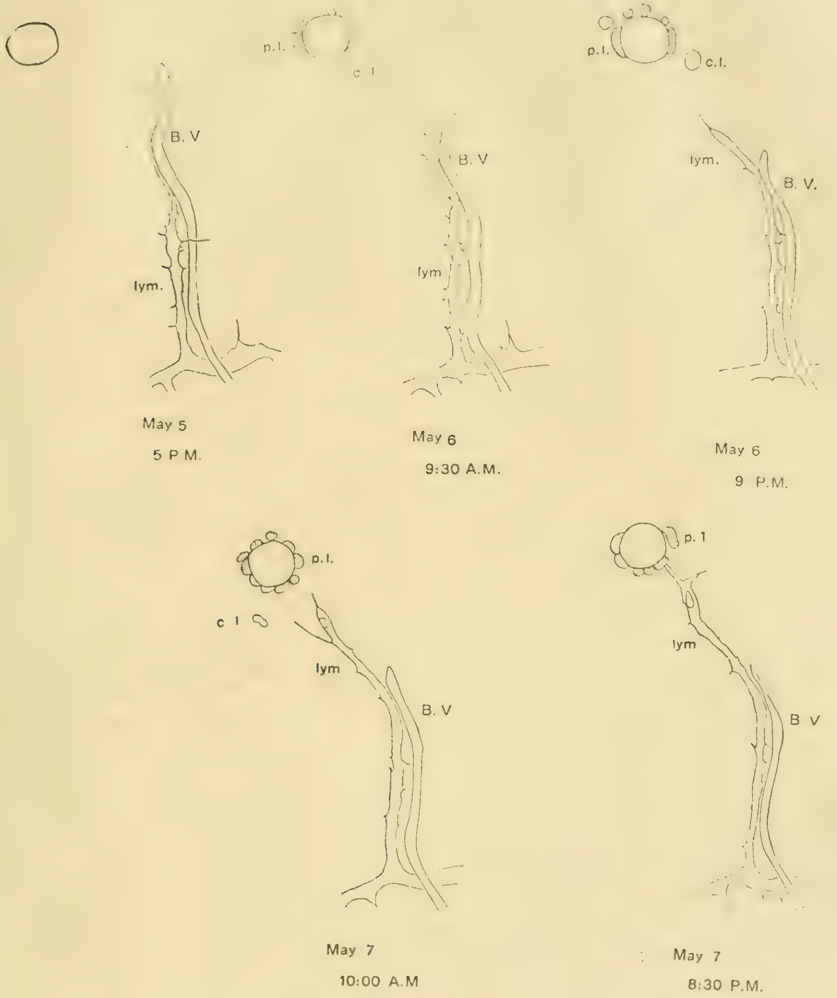


FIG. 2 A series of records showing the reaction of a lymphatic capillary toward an injected globule of olive oil. The first sketch, May 5, was made immediately after the injection. The next drawing, sixteen hours later, shows the globule surrounded by pigmented leucocytes and the lymphatic apparently growing toward the globule. The next two sketches show the lymphatic sprout approaching the globule and in the last one, the lymphatic has reached the oil and the tip is in close proximity to the globule and to the rim of leucocytes. Note that the blood vessel shows no reaction toward the globule but instead it retracts during the observation. *B.V.*, blood vessel; *lym.*, lymphatic; *p. l.*, pigmented leucocyte; *c.l.*, clear leucocyte. Enlargement = 140x. Drawn with camera lucida.

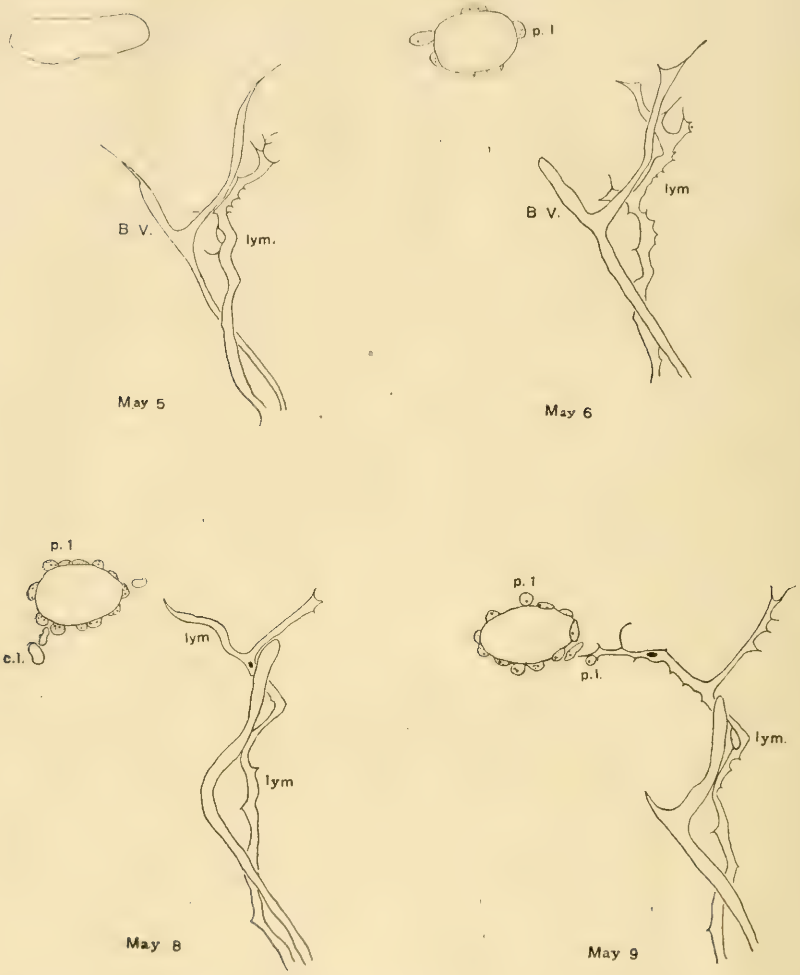


Fig. 3 Another series of records, showing the reaction of a lymphatic capillary toward an injected globule of olive oil. The first sketch, May 5, was made immediately after the injection. The successive sketches show that the lymphatic, although pointing to the right at the time of injection, soon sent out a sprout to the left, which grew toward the olive oil. The sketches also show the retraction of a blood-vessel which was near the globule at the time of injection. *B.V.*, blood vessel; *lym.*, lymphatic; *c.l.*, clear leucocyte; *p.l.*, pigmented leucocyte. Enlargement = 140x. Drawn with camera lucida.

appeared to be larger than the cells which took them in (fig. 6). On successive days, such a globule inside of a leucocyte, diminished in size and simultaneously the minute fat droplets and brown pigment in the cells increased, apparently showing that the oil globule was being resolved into a finer and finer emulsion.

The injected globules of olive oil diminished slowly but steadily in size but they were not completely absorbed at the end of nineteen days.

No reaction toward the injected olive oil on the part of blood-vessel endothelium or mesenchyme cells was noted in any of the observations.

2. Oleic acid. This substance was injected in the same manner and amount as the olive oil. Within a minute or two after coming in contact with the intercellular fluid, the oleic acid changed from a clear refractile globule to an opaque granular mass with irregular margins, which was brown by transmitted and white by reflected light. It was suspected that the oleic acid had been combined to form a soap. In order to be reasonably certain of this, hard and soft soaps were made by boiling oleic acid with sodium hydroxide and potassium hydroxide respectively. The sodium oleate thus produced was found to have the same microscopical appearance as the changed oleic acid, while the potassium oleate differed markedly. It, therefore, seemed fair to assume that the oleic acid is combined with sodium of the tissue fluid to form the soap, sodium oleate, and that, instead of oleic acid, we are studying the reactions of the tissues to its sodium soap.

The leucocytes responded to this substance more quickly and in larger numbers than in the case of the olive oil. They migrated toward the injected mass, formed a ring around it, several layers deep, and soon became deeply pigmented. On the day following the injection, many small refractile droplets were visible near the point where the oleic acid had been injected. These were scattered through the opaque brown mass and in the area around it and superficial to it. Such droplets were not present in other parts of the tail. The leucocytes surrounding the injected mass also contained these refractile drops in addition to the brown pigment. The chemical nature of these droplets was not determined.

The response of lymphatic endothelium to the presence of the sodium oleate was similar to the reaction toward olive oil. Nearby lymphatics sent out processes which extended toward the site of injection, increased in size, acquired a lumen, and finally came into contact with the pigmented leucocytes surrounding the soap (fig. 4). Continuous observations of the region were made and the movements of each leucocyte in the field was followed, by means of drawings made at intervals of about five minutes. It then became evident that pigmented leucocytes were continually moving away from the injected mass and wandering up to a nearby lymphatic capillary. After remaining for

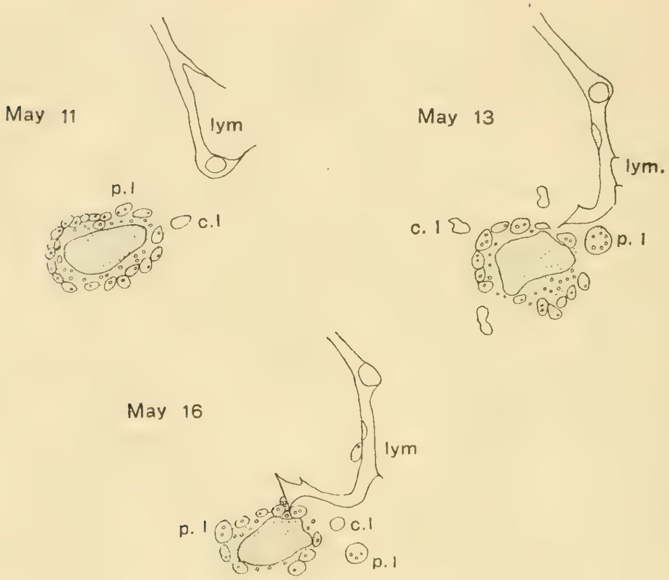


Fig. 4 Series showing the reaction of a lymphatic capillary toward an injected globule of oleic acid (soap). Soon after the injection, May 11, the lymphatic sprout extends in the opposite direction from the injected mass. On May 13, it is shown approaching the mass and in the last sketch it has reached it. *lym.*, lymphatic; *p.l.*, pigmented leucocyte; *c.l.*, clear leucocyte. Enlargement = 187x. Drawn with camera lucida.

fifteen minutes to half an hour or more in close contact with the tip or wall of the lymphatic, the leucocyte would move away again. Shortly before or at the time of wandering away, the leucocyte lost its brown pigment and became clear (fig. 7).

The mesenchyme cells, just as in the case of the olive oil, remained totally indifferent to the presence of the injected substance. In no instance did the blood-vessel endothelium respond to the fatty acid or soap by growing toward it or by sending out a process. However, in one set of observations in which the brown injected mass, with its sprinkling of refractile droplets and its circle of pigmented leucocytes, had shifted its position so that it came to occupy a position very near to a blood-vessel, leucocytes, containing pigment and droplets were seen, on three successive days, crawling around the wall of the blood capillary. Although they were watched continuously for several hours, they were not seen to lose their pigment. This was the only instance, in all the experiments, in which there was a suggestion that the neighboring blood capillaries might play a part in the absorption of the injected fat.

The absorption of the oleic acid, or sodium oleate, proceeded more quickly than in the case of the olive oil but was not complete after ten days. This substance changed its shape and shifted its position much more noticeably than the olive oil.

3 and 4. Cream and *yolk of egg*. Since these two substances were similar in appearance, upon injection, and produced identical results, a single description will suffice for them both. A very small amount of each substance was injected into the subcutaneous tissue of the tail. Under the binocular microscope, the injection appeared as a line of black dots; viewed under the compound microscope, both the cream and yolk were seen to consist mainly of an emulsion of very small fat droplets.

Soon after the injection, many leucocytes could be seen crawling through the walls of neighboring blood capillaries and moving toward the yolk or cream. Upon reaching the injected substance, these cells proceeded to ingest the fat droplets. Soon after taking up the drops of cream or yolk, the leucocytes became pigmented, owing to the numbers of very small granules which they contained, the larger of which were refractile and indistinguishable from the droplets composing the emulsion.

When continuous observations of the injected region were made together with records drawn at five to ten minute intervals, it was found that pigmented leucocytes migrated from the injected mass and approached the tip or wall of a nearby lymphatic capillary. Here they remained for from five to ten minutes closely adherent to the lymphatic and then moved away. Just before or at the time of wandering away again, the leucocytes lost their pigment and, in most cases, became quite clear (fig. 8). This reaction of the pigmented leucocytes to lymphatics was similar to that described for the other two injected substances, but here the process was completed in a shorter time than in the case of the oleic acid and much more rapidly than in the case of the olive oil. In those larvae in which the yolk of egg or cream had been introduced near a lymph capillary, leucocytes containing granules and droplets approached the lymphatics within two or three hours following an injection. When the substance was injected at some distance from a lymph vessel, the pigmented leucocytes remained in a thick cluster at the injection site for ten to twenty-four hours and then many of them wandered away, often moving toward a growing lymphatic sprout. In such cases, most of the leucocytes lost their pigment before coming in contact with a lymphatic capillary.

The absorption of cream and of yolk of egg took place extremely rapidly: twelve hours after the injection, most of the droplets had been taken up by leucocytes and at the end of twenty-four hours, all of them had been. On the second day after the injection, only a few pigmented leucocytes were present at the point of the injection (figs. 5 and 9).

On account of the rapidity of the absorption, the response of lymphatics toward the injected cream or yolk was not so striking as in the case of the substances which were absorbed more slowly, since in many cases, the leucocytes had taken up all of the fat droplets and many of them had started to migrate toward the lymphatics before there was time for any marked

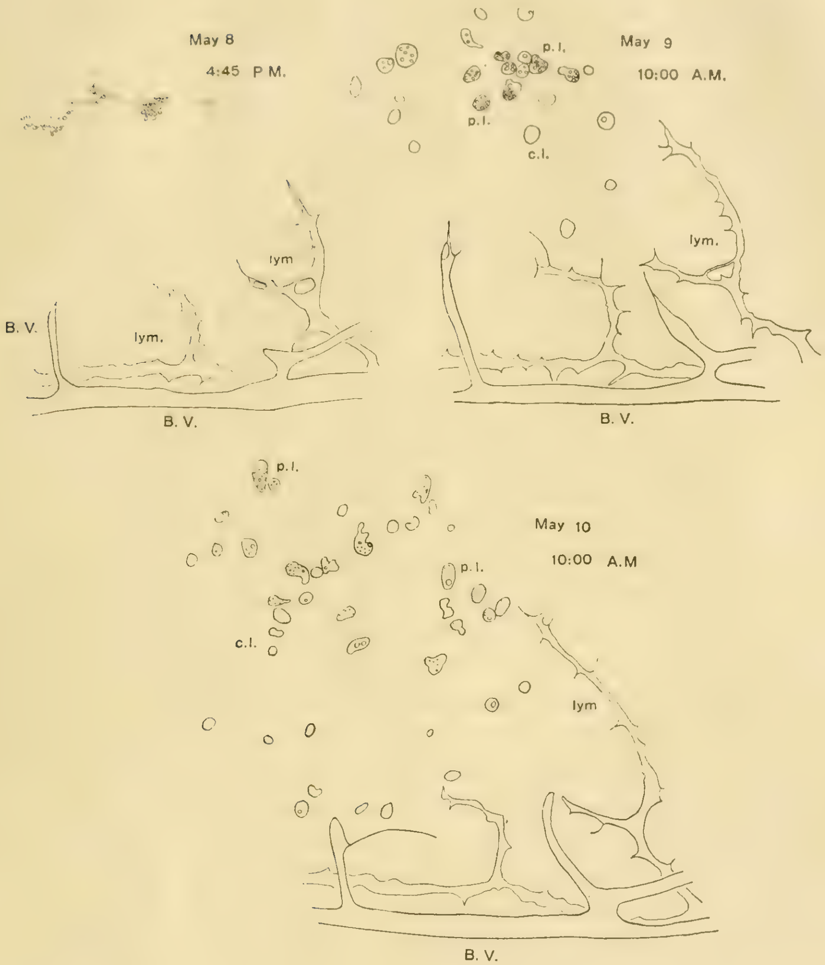


Fig. 5 Illustrates the reaction of lymphatic capillaries toward an injection of cream. The first sketch was made immediately after the injection. The second, made seventeen hours later, shows that the small globules of cream have all been taken up by leucocytes. One of the lymphatic capillaries is seen to have grown, somewhat rapidly, toward the site of injection. In the last drawing, the lymphatic has extended still further and the pigmented leucocytes are wandering off, a number of them in the direction of the growing lymphatic sprout. As in figure 2 the blood vessel sprouts show no definite reaction toward the injected substance. *B.V.*, blood vessel; *p.l.*, pigmented leucocyte; *c.l.*, clear leucocyte. Enlargement = 140x. Drawn with camera lucida.

growth on the part of the lymphatic endothelium. However, in experiments in which the site of the injection was located at some distance from the lymphatic, a definite growth of lymphatic sprouts toward the fat was observed and this growth continued after all of the injected droplets had been engulfed by leucocytes (fig. 5). When one of these rapidly absorbable emulsions was injected near the tip of a lymphatic, as in the series shown in figures 8 and 9, the lymphatic sent out a fine process in the direction of the nearest pigmented leucocytes.

Blood vessels and mesenchyme cells were never observed to react in any way to the presence of the cream or yolk of egg.

In comparing the results obtained from the foregoing experiments, it is evident that the reaction of the cells and tissues of the tad-pole's tail was essentially the same in the case of all the substances tried. Any difference in response was one of degree and not one of kind. In general, the presence of the injected fat called forth a response on the part of two types of tissue—lymphatic endothelium and leucocytes. Connective tissue cells did not react, in any observable manner, even when the injected globule or mass of droplets pressed against them. Although it is not impossible that the blood capillaries may have taken some part in the absorption of some of the soluble products of the lipolysis, the blood-vessel endothelium did not send out sprouts or processes nor show any visible reaction to the presence of the fatty substance. Since the reaction of living cells and tissues, to substances selected for the experiments was confined to lymphatics and leucocytes, and since fat injected subcutaneously appeared to be absorbed through the combined efforts of these two types of cells it is desirable to give a more detailed account of the reaction (1) of lymphatics and (2) of leucocytes, toward the injected fat.

THE REACTION OF THE LYMPHATICS

Lymphatic endothelium reacted positively toward all of the fatty materials used in these experiments. When the substance had been injected near a lymphatic capillary, a fine pointed process was sent out from the lymphatic, which extended in the direction of the fat and soon came into contact with the surrounding leucocytes. When the rapidly absorbed emulsions of cream and yolk of egg were the substances employed, this sending out of fine processes was the only noticeable growth reaction on the part of the lymphatic. On the other hand, when the injected material happened to be olive oil or oleic acid, the new processes of the lymphatic persisted longer and acquired a lumen. Once, a forked process was sent out which enlarged, acquired a lumen, and extended on either side of the oil, in close contact with the rim of leucocytes, as two branches of the lymphatic.

When the injected substance was made at a distance from a lymphatic, the reaction was much more striking. A finely pointed process was sent out from a nearby lymphatic, which grew out in the direction of the injected fat. This process increased rapidly, in length, acquired a lumen and grew further, soon outdistancing the other lymphatic and blood vessel sprouts of that part of the tail. In case the substance injected did not chance to be one of the rapidly absorbable emulsions, this new lymphatic capillary grew directly toward the site of injection and came into close contact with the oil or soap and its surrounding leucocytes (fig. 2). In figure 2 is given a series of records illustrating the growth of a lymphatic sprout toward an injected globule of olive oil. It will be seen that the lymphatic tip grows directly toward the oil and reaches it two days after the injection, having grown a considerable distance during that period. In the first record, made immediately after the injection, the lymph capillary is seen to be much shorter than the neighboring blood capillary. In the last record, made after the lymphatic has reached the globule, the lymph sprout is almost twice as long as the blood vessel, the latter having retracted during the observation.

In another series of observations (fig. 3) a globule of olive oil was injected to the left of a lymphatic and some distance away. A drawing, made immediately afterward, showed that the tip of the lymphatic was pointed toward the right and away from the oil globule. A blood vessel was present at the left of the lymphatic and one of its main branches extended nearly to the injected globule. A record made eighteen hours later showed that, from the tip of the lymphatic, a new branch had been sent out to the left, on the side toward the oil globule, which had already extended some distance in the direction of the oil globule. Three days after the injection, this newly formed lymphatic sprout had reached the pigmented leucocytes surrounding the oil globule. While this sprout, during its growth toward the oil globule sent out a branch in the opposite direction, its uninterrupted extension to the oil indicates, pretty definitely, that the presence of the oil exerted on it an attractive influence. The blood vessel sprout, in the meantime, retracted steadily. This retraction may have had nothing to do with the presence of the oil, as such new blood vessel sprouts have often been observed to retract. Considered, however, in connection with the record shown in figure 2, it is suggested that possibly the oil exerts a repellant influence on blood-vessel endothelium.

The attraction of injected fatty substances for the lymphatic endothelium continues after the fat droplets have all been taken up by leucocytes, since in the case of the cream and yolk, injections, made at some distance from a lymphatic, a sprout continues to grow toward the site of injection when extra-cellular fat is no longer present. In such cases, many of the pigmented leucocytes scatter, part of them moving toward the lymphatic. Some become clear during the migration and it is probable that the soluble, and therefore invisible, substances, set free from the leucocytes, exert this attraction on the lymph sprout. It should be mentioned in this connection, that even in its response toward the more slowly absorbed substances, the lymphatic capillary comes into contact with the pigmented leucocytes surrounding the olive oil, or sodium oleate, rather than with the fatty substances themselves.

The manner in which the lymphatic reacts toward these various substances is very similar to its response toward the extruded red blood cells, described in 1909 (3), and referred to earlier in the present article. In both cases, the lymphatic sent out fine processes toward the external stimulus present in the form of fat or of erythrocytes and when this stimulus was situated at some distance, these processes enlarged, acquired a lumen and grew further. In the process of taking up of red blood cells, the lymphatic is actively phagocytic; in the taking up of the fat, the lymphatic is assisted by leucocytes and appears to absorb the substance in a soluble form. Otherwise, the two reactions are identical and both are exactly similar to the normal growth processes for lymphatic capillaries, which have been described fully in previous communications, the only apparent difference being that in the present experiments, as well as in the observations on the taking up of red blood cells, the stimulus to growth is a visible one. These experiments seem to add new weight to the hypothesis, proposed in 1909 (3), that the growth of lymphatic capillaries, after their primary differentiation, is inseparably connected with their function (i.e., with absorption) and that it is the varying formation of certain substances in the tissue spaces which regulates the growth of the lymphatic capillary.

As to the precise nature of the reaction; whether the lymphatic endothelium is attracted by the specific chemical substances, that is, whether they exert a positive 'chemiotactic' influence on the lymphatic, or whether it is mainly a response of the lymphatic to the quantity of substances moving toward it and being absorbed, is impossible, on present evidence, to decide. The fact that blood vessel endothelium failed to grow toward the injected fatty substances, while lymphatic endothelium did the reverse, is in favor of an especial relationship between these substances and lymphatic endothelium. In all probability, it will be found that it is both a qualitative and quantitative reaction; that certain groups of substances, among them the fatty substances used in the present experiments, are attracted to and absorbed by lymphatics, and that extension of the lymphatic takes place when the rate of absorption exceeds a certain point. It is hoped that further experiments will help to answer some of these questions.

THE REACTION OF LEUCOCYTES

The behavior of the leucocytes was essentially the same toward each of the various substances employed. Figure 1 illustrates the initial response of leucocytes toward the injected material. These cells migrate from nearby blood-vessels and move toward the injected fat and within five or ten minutes after reaching it, they are seen to contain refractile droplets and brown granules. In the case of the single relatively large globules of olive oil, the leucocytes formed a ring around the oil consisting of a single layer of cells. In the case of the soap of oleic acid, the leucocytes penetrated the substance for some distance and remained as several layers in the less compact peripheral portion of the mass. In the case of the yolk of egg and cream, leucocytes wandered toward the injection site and actively engulfed the small fat droplets composing the respective emulsions.

In all of the substances used for the experiments, leucocytes containing refractile droplets and brown granules, were observed to wander away from the site of injection and to move toward a nearby lymphatic capillary. After coming into close contact with the wall or tip of the lymphatic, they remained there for varying lengths of time (five to ten minutes in the case of the yolk and cream, about twenty minutes in the case of the oleic acid, and as long as ten or twelve hours when they contained drops of olive oil). This relationship of leucocyte to lymphatic is shown in figures 6, 7 and 8. At first sight, it often seemed that such a leucocyte was just upon the point of entering the lymphatic. However, when the region was observed closely over a sufficient period of time, the leucocyte was always seen to wander away. In fact, no pigmented leucocytes were seen to enter a lymphatic during the course of any of the observations. At the time of wandering away, the pigment within these phagocytic cells either disappeared entirely or diminished perceptibly in amount (figs. 7 and 8).

From these observations alone it was of course impossible to determine the exact chemical process by which the various fatty substances were absorbed. As an interpretation of these records of the living cells and their reaction toward the various substances, it can be stated that leucocytes migrated toward the site of injection and actively phagocytized the fat in the form of small droplets and that subsequently the fat within the cells apparently became split up into a finer and finer emulsion (the brown pigment granules) and that, eventually, it was changed to a soluble form, in which state it was absorbed by the lymphatic capillaries.

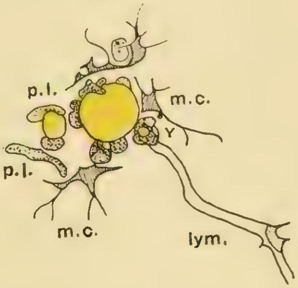


Fig. 6 Illustrates relationship of leucocytes to lymphatics during the absorption of a globule of olive oil. Oil injected May 5. This drawing made May 15. This is the same specimen and oil globule as that shown in figure 2. The globule is surrounded by pigmented leucocytes some of them containing small oil globules. The smaller globule to the left has become separated from the main globule. The oil is represented by the color. *lym.*, lymphatic; *m.c.*, mesenchyme cell; *p.l.*, pigmented leucocytes; *y*, pigmented leucocyte, closely adherent to the tip of lymphatic. Enlargement = 187x. Drawn with camera lucida.

This characteristic mode of response toward injected fat on the part of the leucocytes of the tadpole's tail, stands in marked contrast to the behavior of the same type of cells toward the injected globules of paraffin oil. In the case of the experiments with paraffin oil, as described in the earlier article (5), leucocytes collected around the oil soon after its injection. This leucocytosis was always transitory. In some cases in which this temporary reaction was intense, probably as a result of infection, the globule was extruded. In most instances, the leucocytosis had subsided, after three or four days and, in all cases, at the end of a few days more. When individual leucocytes were

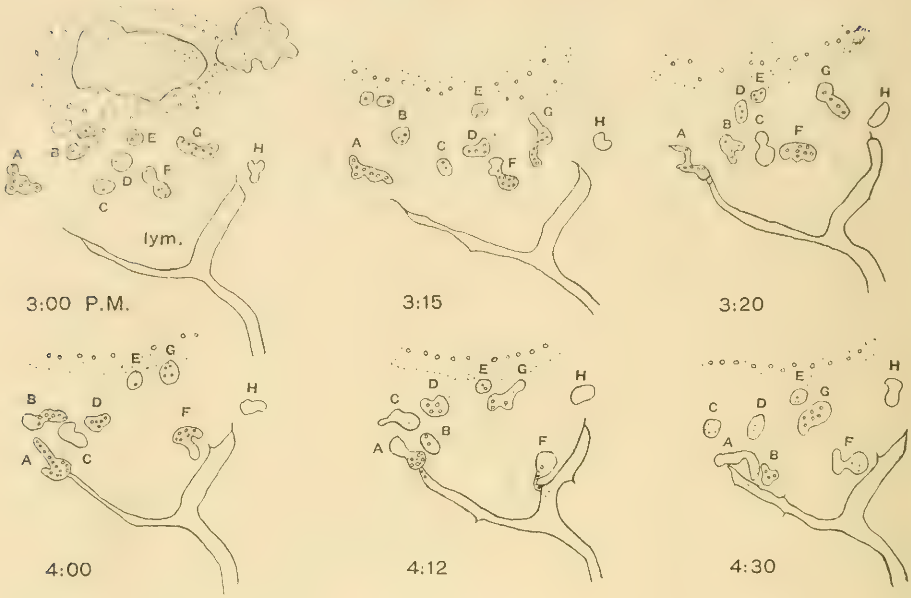
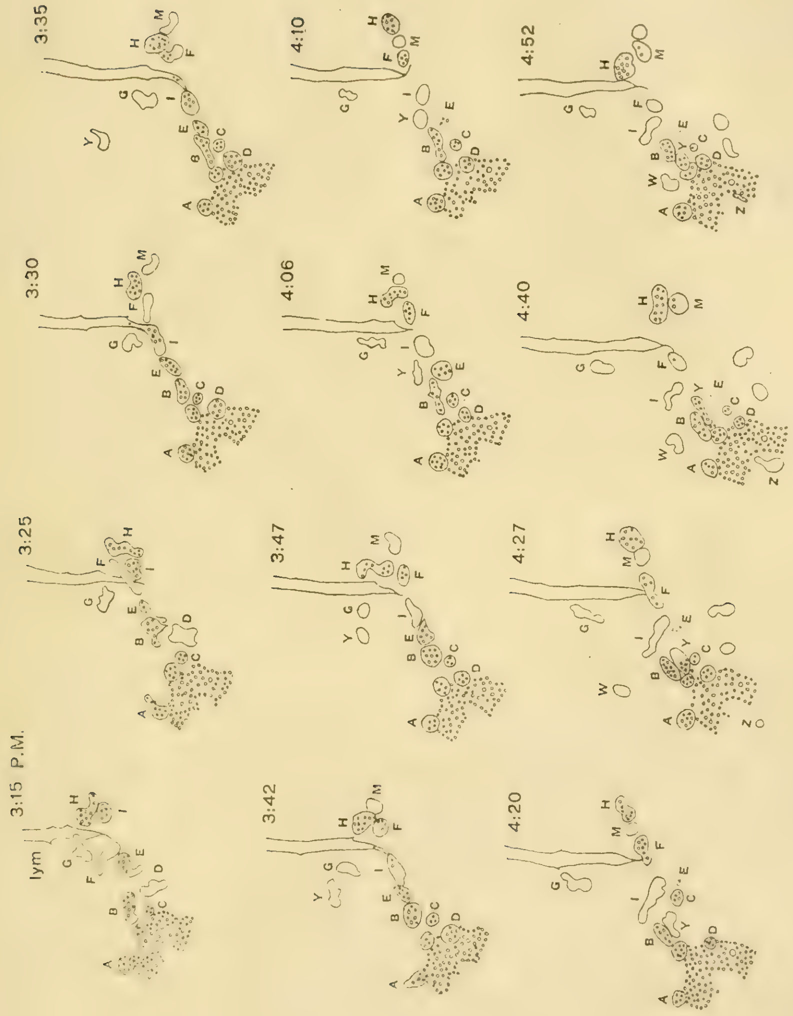


Fig. 7 A series of drawings showing the relationship of pigmented leucocytes to lymphatics during the absorption of an injected globule of oleic acid (soap). The globule was injected on May 17. The series of records were made May 18. The opaque mass of soap is shown only in the first sketch. Leucocytes *A* and *F* were seen to move away from the injected mass and to approach the lymphatic. After remaining closely attached to the lymphatic for varying lengths of time, they were seen to move away. Just before moving away they became clear. In each case, as the leucocytes lost their pigment, a very few small granules were seen inside the lumen of the lymphatic which had not been previously noted. *lym.*, lymphatic. Enlargement = 187x. Drawn with camera lucida.

Fig. 8 A series of records showing the relationship of leucocytes to lymphatics during the absorption of a small amount of yolk of egg. The first record shown here was made three hours after the injection. The yolk of egg was injected near the tip of a lymphatic sprout. Pigmented leucocytes *I* and *F* became attached to the tip of the lymphatic and remained there for about ten minutes, and then moved away. Just before wandering away from the lymphatic they became clear. Clear leucocytes *D*, *Y* and *Z* are shown approaching the injected mass and taking up the small yolk globules. Leucocytes *F* and *M* became pigmented after close contact with leucocyte H, which contained a large amount of pigment and granules. Leucocyte *E* disintegrated during the observation. *G* remained in approximately the same position throughout the observation. *lym.*, lymphatic. Enlargement = 168x. Drawn with camera lucida.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lym
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A
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watched closely and records kept, they were seen to approach the globule and sometimes to flatten out on its surface and then to move away again.

In the present study, we confined our attention to the study of the living. It would be of interest to study the leucocytes, containing fat, in fixed specimens and with special stains in order to discover if this reaction is confined to any one type of leucocyte. That such might be the case was suggested in several of our series of observations, owing to the fact that certain clear leucocytes, favorably located with respect to the injected fat, often remained in the same position, occupied at the time of

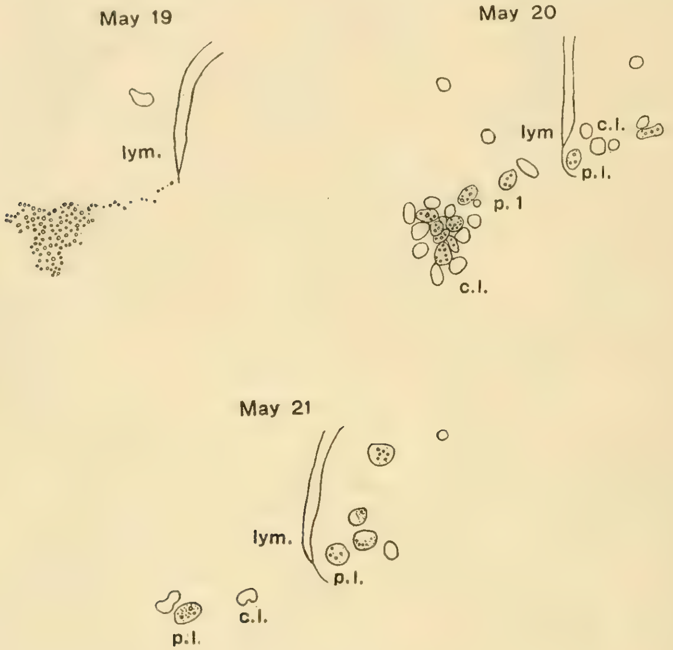

Fig. 9 Drawings of the same specimen and injection mass as in figure 8, showing the rate of absorption of an injected mass of small globules (yolk of egg). May 19, sketch made immediately after the injections. May 20, all of the small globules have either disappeared or are found inside of leucocytes. May 21, only a few pigmented leucocytes, containing a few granules of yolk, remained at the injection site. *lym.*, lymphatic; *p.l.*, pigmented leucocyte; *c.l.*, clear leucocyte. Enlargement = 187x. Drawn with camera lucida.

injection, while others came out of blood vessels at some distance away, moved past these leucocytes and finally reached the fat. Such a leucocyte which did not react to the presence of the fat is illustrated as *G* in figure 8. It remained in practically the same position throughout the observations, although it was much nearer the injection site than several other leucocytes, such as *Y*, upon which the fat exerted an attraction. An investigation of this question would be of interest in view of the discovery of Fiessinger and Marie (10) that lymphocytes contain lipase, while polymorphonuclear leucocytes contain the protein splitting enzyme.

The present studies were completed in the spring of 1916, before the work of Wislocki (28) had been published, and were made without the aid of trypan blue. In the study published in 1909 (3), the results of staining tad-poles with neutral red was described, and neutral red was used in the present experiments, also. This stain is not specific in its reaction since it stains the epithelium intensely and all tissues rather faintly. Nevertheless, on focussing beneath the epithelium upon the field of observation for the experiments, it was noticeable that the red granules of the stain were confined almost wholly to two types of cells, namely, to the endothelial cells of the lymphatic capillaries and to the pigmented leucocytes. A very few red granules were scattered along some of the blood capillaries, but in the lymphatic endothelium, the stain was quite dense and had collected in clumps which were located chiefly in the perinuclear areas. Pigmented leucocytes, which had taken up the stain, were scattered at various points through the fins, and their presence was much more conspicuous, on account of their numbers and the intensity of their staining, in the region where the fatty substance had been injected. It would be interesting to determine whether or not these leucocytes, which show a special affinity for the injected fat, would also stain with trypan blue—to discover whether or not they too should be classed as 'pyrrhol cells.'

DISCUSSION OF THE RESULTS IN CONNECTION WITH THE QUESTION OF FAT ABSORPTION

In the present investigation we were concerned chiefly with the question of the reaction of the growing lymphatic toward an external stimulus, which in this case was represented by the injected fat. Therefore, the scope of these experiments did not enable us to deal with the problem of fat absorption except in an incidental manner.

According to a recent summary by Bloor (2), most investigators now agree with the findings of Pflüger (22, 23) that fat in the small intestine is saponified through the action of the pancreatic lipase and the bile salts and is absorbed by the intestinal mucosa in the form of water-soluble soap and glycerol. However, Rossi (25) and Levites (18) claim that part at least of the fat is absorbed in the form of fatty acids. The work of Kastle and Loevenhart (15) on the reversible action of lipase on ethyl butyrate, which has been confirmed by Pottevin (24) with regard to the higher fats, and the discovery of Loevenhart (19) that lipase is a normal constituent of the epithelial cells of the intestinal mucosa, make it clear that the products of the lipolysis are resynthesized within the intestinal cells. Sixty per cent of the absorbed fat is known to be taken up by the lymphatic system and reaches the thoracic duct in the form of neutral fat. The rest has been shown by Hamburger (12), by D'Errico (7) and by Mendel and Daniels (20) to be conveyed to the liver by means of the portal circulation. The fat remains within the blood, suspended in a finely divided state, for about two hours after intestinal absorption and then disappears. Bloor (1, 2) has shown that the fat in the blood is taken up, to a large extent, by the blood corpuscles, both red and white, and there transformed into lecithin. Leathes (17) investigated the later stages of fat absorption and has shown that the fatty acids are desaturated in the liver in prepartion for the process of oxidation.

The mechanism by which the fat which is resynthesized in the intestinal mucosa, reaches the central lacteals of the villi, has not been clearly demonstrated. Loevenhart (19) advanced the theory, based upon his observations of the reversible action of lipase, that the neutral fat, present in the intestinal cells during fat absorption, represented merely a stable phase in the reaction and that the absorbed fat was continually leaving the intestinal cells and entering the lymphatics in a soluble form, probably fatty acids, and was resynthesized somewhere in the lymphatic system (Loevenhart had previously shown that lipase is present in the lymph and the lymph glands). B. Moore (21) questions this hypothesis on the basis of analyses which showed that practically all the fat in the lacteals during absorption exists in the form of neutral fat, while the intestinal mucosa, from the same animals, yields a much larger amount of fatty acids.

For many years, investigators, who have studied this question by histological methods, have described leucocytes as taking part in fat absorption. Thus, Zawarkyn (29) in 1883 found many leucocytes present in the intestinal villi, during fat absorption, containing granules which stained with osmic acid. In 1885, Schäfer (27) described similar cells as playing an important rôle in the process of fat absorption. His theory was that leucocytes, loaded with fat, entered the central lacteals and there disintegrated and set free the fat. Rachel Zipkin (30), in studying the histology of the intestine in the monkey, found many phagocytic cells, closely adherent to the wall of the central lacteals of the intestinal villi. D'Errico (7) describes large numbers of leucocytes containing fat in his experiments in which he ligated the thoracic duct during intestinal absorption. Kischensky (16), in studying the intestinal epithelium in preparations stained with Scharlach R and osmic acid, found many finely divided droplets inside the epithelial cells, during fat absorption, and also between the cells. He also found fat within leucocytes during fat absorption in young kittens. In these cases the fat was present chiefly in small round cells with large nuclei and was seldom found in polymorpho-nuclear leucocytes. This last point is of interest in connection with the work of Fiessinger and Marie (10) on exudates, which has been referred to earlier in this article, in which they demonstrated the presence of lipase in lymphocytes and showed that the polymorpho-nuclear leucocytes contain a proteolytic ferment. It is justifiable to conclude from all of the foregoing observations that leucocytes play some part in the absorption of fat.

The observations reported at this time, on the reaction of living cells to fat injected subcutaneously, showed that leucocytes are strongly attracted toward the various fatty substances selected for these experiments, that they phagocytose the fat and usually convey the engulfed fat to a nearby lymphatic, and that they transform the fat intracellularly into a soluble substance or substances which are absorbed by the lymphatic. The chemical constitution of the products of this lipolysis obviously could not be determined by the present method of study. And it is, of course, impossible to state how far the results of these observations are applicable to the question of fat absorption in the intestine.

Another point which came out strikingly in the present experiments is that the rate of absorption of fat injected subcutaneously is dependent upon its physical state, that finely divided emulsions are taken up and disposed of much more quickly than those substances which exist as single globules of oil.

The very rapid transformation of oleic acid immediately after its injection into what was probably sodium oleate has been described. Just what element in the tissue fluid caused this reaction cannot be stated. We also are not prepared to explain the nature of the small refractile droplets which made their appearance through and around the opaque mass of soap on the day following the injection. Frank and Ritter (11) have shown that carbon dioxide will cause the splitting of fatty acid from soap, in vitro, and perhaps this may be the explanation of this phenomenon.

SUMMARY OF RESULTS

a. Lymphatic endothelium, in the tail of the frog larva, reacts positively toward the fatty substances, olive oil, oleic acid, cream and yolk of egg, injected into the tissue spaces, by sending out sprouts which grow to or toward them.

b. Leucocytes respond quickly to the injected substances, migrate toward them in large numbers and actively engulf the fat.

c. The fat is absorbed, apparently, through the combined efforts of leucocytes and lymphatics.

d. Mesenchyme cells and blood-vessel endothelium are unaffected in any observable way by the presence of the injected fat.

e. The rapidity of absorption depends upon the size of the fat droplets: the fine emulsions of cream and yolk of egg are taken up very much more quickly than the single relatively large globules of olive oil and oleic acid.

f. The fat appears to be changed within the leucocytes and to be absorbed in a soluble form by the lymphatics.

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心臓瓣膜ノ血管

スタンホープ、ベイン＝ジョーンス述

本論文ハ冠狀動脈ヲ通ジテ心臓ニ注射ヲ施セル結果ヲ報告シ多年ノ論題タリシ心臓瓣膜内血管ノ有無ヲ確カメントセルモノナリ。　豫備試驗トシテ豚心臓ニ就テ實驗シ其瓣膜ガ血管ニ富メルコトヲ知レルガ、注射ニヨリ此血管ヲ證スルニハ、及ブ限リノ高壓ヲ用ヰザルベカラズ。　著者ノ用ヰタル注射劑ハ一・五％グリューブラー「かるみん」及一五％「ぜらちん」ノ中性合劑ニシテ注射中ハ攝氏四十五度ノ溫度ニ保タシメ管ノ嘴端ヲ冠狀動脈内ニ挿入シ之ヲ加壓裝置ニ連結シ水銀柱百四十乃至百九十粍ノ壓ヲ與ヘタリ。　凡テノ靜脈及心臓表面ノ創傷ハ豫メ縫合結紮ヲ施シテ注射劑ノ漏ルルヲ防ギ注射中ハ臓器ヲ攝氏五十度ノ鹽溶液中ニ置キタリ。　然ル後瓣膜及注入セル「かるみんぜらちん」ヲ「ふおるまりん」ニテ固定シ、スパルテホルツ氏法ニ從ヒ「うぃんさーくりーん」油及「べんずる」中ニテ透明ナラシメタリ。

正常人心臓十四例ニ該法ヲ施セルニ凡テニ就テ瓣膜中ニ血管アルコトヲ證シ得、又特ニ三例ニ於テハ房室間瓣膜及半月狀瓣膜内ノ血管造構ニ就テ極メテ完全美麗ナル標本ヲ得タリ。　房室間瓣膜内ノ血管ハ冠狀動脈環狀枝ヨリ派出シ、半月瓣膜ノ血管ハ心上部血管並ニ肺動脈及大動脈隨伴血管ヨリ來ルコト、又腱索内ニモ血管ノ存在スルコトヲ知ラレタリ。　此結果ハ心瓣膜内ニ血管ヲ發見セル從來ノ解剖學者ノ學說及業蹟ヲ增補シ且心内膜炎ノ病因ガ栓塞ニ基クコトアリトノ說ニ一根據ヲ與フルモノナリトス。

THE BLOOD-VESSELS OF THE HEART VALVES

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SIX TEXT FIGURES AND ONE PLATE

The interest of pathologists in the study of the process of bacterial disease of the heart-valves has stimulated many contributions to the knowledge of the anatomy of these structures. It is important for the pathologist to know whether bacteria become localized in the heart-valves by mechanical adherence to their surfaces or by lodgment within the tissue of the valve. The latter view, which served as the basis for Köster's (1) theory of 'Embolic Endocarditis,' is founded upon the supposition that there are blood-vessels within the valves. Anatomical studies to determine whether or not blood-vessels are present in normal heart-valves have led to such various findings that the subject has been in a state of prolonged controversy. During the past, however, there has been gathered enough positive evidence of vascularity of the valves of the heart to outweigh the negative results of investigators who have failed to find these vessels. The studies to be reported in this paper confirm the work of those who have described the blood supply of the heart-valves and in addition provide histological details of the vascular arrangement not hitherto noted.

HISTORICAL

The following critical review of the important writings on the subject of the vascularity of the heart-valves indicates the development of the controversy:

Luschka (2) was the first pathological-anatomist to state that blood-vessels occur in the heart-valves. In 1852 he published an article illustrated with pictures of valves after injection of the blood-vessels of normal hearts. Luschka found a network of vessels in the broad part of the valve with small vessels running to the line of closure, and a few vessels in the lower portions of the chordae tendineae. In the 1863 edition of his 'Anatomie' (3), Luschka states that blood-vessels are present in the semilunar as well as the auriculo-ventricular valves of the heart, and he illustrates this chapter with drawings from injected specimens of normal valves. He describes both arteries and veins in the valves and suggests that endocarditis may be related to the presence of these vessels. He noted that it was easiest to find vessels in the aortic leaflet of the mitral valve and correlated this finding with the great frequency of endocarditis lesions on this part of the valve. Luschka's drawings, however, do not show the details of the relationship between the various vessels and they are incorrect in that they indicate that the capillaries end blindly in the valves. As will be shown later, there are definite vascular anastomoses here and a characteristic capillary bed. It is probable that Luschka's injection mass did not penetrate sufficiently far.

After Luschka's clear presentation of the subject, Joseph (4) immediately opposed the idea that blood-vessels are present in the valves. Since then the controversy has continued. The difficulty in demonstrating the presence of these vessels seems to have been the chief subject matter of the criticism of Luschka's findings. It is obvious that Joseph did not inject the valves as Luschka did. By some prescience, whose source is not in the writings of Luschka, many authors decided that the valves studied by Luschka were abnormal.

Among the influential opponents of Luschka were Coen (5) and Langer (6). These authors found that the atrio-ventricular valves were partially vascularized, while normal semilunar valves never contained blood vessels. Langer associated the presence of vessels in the atrio-ventricular valves with the occurrence of fibres of smooth muscle. By making this correlation, he announced a doctrine which has been invoked frequently since then. Practically all subsequent studies have shown that the blood-vessels are fairly abundant whenever there are muscle fibres in the valves. These are supposed to be confined to the basal third of the valve, and it is probably the rule that in the adult these structures are limited to this region. Numerous studies of the musculature of the valve have been made. In Tandler's (7) recent "Anatomie des Herzens," the situation is summed up as follows:

> Corresponding to their developmental process, the atrio-ventricular valves of the embryo contain considerable muscle and a corresponding number of blood-vessels. This musculature has a close relationship to the auricular musculature, so that in fetal hearts and those of young children, vessels are seen extending as far as this musculature. In the fetal heart, and in the young heart, vessels extend from the papillary muscles to the chordae tendineae and along through to the valve. The vessels diminish along with the muscle fibres of the chordae and valves. The almost muscle-free semilunar valves are also practically devoid of vessels. When islands of muscle tissue remain in the thin portions of the valves, delicate blood vessels can be shown to be present here.

In 1903, Königer (8) published an elaborate monograph on endocarditis. His studies of the normal anatomy are mostly literary, as he admits on page 23, he has not attempted to inject normal heart-valves. He grants the vascularity of fetal valves and acknowledges that hematomata and hemangiomata are found at times in the normal valves of new born and young children. He attributes these conditions to vascular rests in the valves, and accepts Langer's position on the absence of blood vessels in adult valves.

The more recent studies of Nussbaum (9) are more convincing. He succeeded in finding blood-vessels in the atrio-ventricular valves of new born, child and adult. Figure II on page 465 is a sketch of the vascular network in the membranous portion of a mitral valve. He was not able, however, to inject vessels in the semilunar valves or chordae tendineae.

The experimental endocarditis produced by Rosenow (10) by intravenous injection of bacteria give a more pathological corroboration to the findings of the anatomists who have demonstrated heart-valve vessels. After injection of streptococci, pneumococci and staphylococcus albus, in rabbits, Rosenow found subendothelial clumps of bacteria in the heart valves, with hemorrhages within the structure of the valve. Rosenow thinks that the localization of bacteria in the valve must depend upon the presence of small blood-vessels there, which become embolized by clumps of bacteria.

The most recent summary of the controversy has been placed by MacCallum (11) in his "Text Book of Pathology." His own studies of the subject make him take position with Langer—limiting the vessels to the basal third of the atrio-ventricular valves. MacCallum states: a "very complete injection of the dogs' heart can be made by clamping the aorta and injecting India ink into the carotid before the heart stops beating." My experience has been that the heart of the dog is not good material to use for such a study, and that an injection mass pushed by the pressure of a failing heart-beat has entirely too slight a force to penetrate the vessels of the heart-valves. Proper pressure, suitable injection mass and the study of cleared specimens are the factors to which I attribute the findings to be recorded here.

EXPERIMENTAL STUDIES

The method of demonstrating the blood-vessels in the heart-valves was based upon the injection of a solution of carmin-gelatin into the coronary arteries. In some cases, as Dr. M. C. Winternitz has pointed out, vessels are visible in the valves of hearts opened at autopsy. It is a relatively simple matter to insert a capillary glass pipette into these vessels and to inject their fine ramifications with India ink. In this way, however, lymphatics are not distinguishable from blood-vessels. Injections through the coronary arteries, on the other hand, give unequivocal evidence of the type of vessel thus filled with the colored mass.

The *carmin-gelatin* used in these injections was prepared as follows:

Gelatin: a 15 per cent. solution of gelatin (gold label) was made by slowly dissolving the requisite amount of gelatin in distilled water over a steam bath. When this was thoroughly dissolved, it was filtered several times through double thicknesses of cheese-cloth.

Carmin: (Grübler's preferred). Sufficient carmin to make 1.5 per cent solution when added to the gelatin solution was pulverized in a mortar, while enough water was added to make a thick paste. The carmin was then dissolved in ammonium hydroxide of known strength. The ammoniacal solution of carmin was then quickly poured into the warm solution of gelatin and thoroughly stirred. To this mixture was added slightly less than the calculated amount of acetic acid required to neutralize the ammonium hydroxide. The color of the carmin-gelatin should be dark red, almost like the color of venous blood. A light red color indicates that too much acid is present, and the carmin will be found in small granules when a drop of the fluid is examined under the microscope. If the mass is slightly alkaline, the dye will diffuse through the injected tissues. The final adjustment of the reaction of the mixture was made from data obtained by titrating small amounts of it against the solutions of ammonium hydroxide and acetic acid. The sweetish odor of the mixture when it is neutral, supplanting the acid or ammoniacal odor of the first stages of the preparation was found to be as valuable as any other indicator of the optimum reaction of the mixture. When neutral, the mixture was filtered through double thicknesses of cheese cloth After the addition of a few crystals of thymol, the carmin-gelatin was stored in flasks until needed. To prepare this for use, it was melted on a water-bath, and kept at 45°C. during the injection

The preliminary experiments were performed upon the hearts of pigs, one or two years old, obtained from the slaughter house. The heart-valves and chordae tendineae of these animals are supplied with vessels which, in microscopic sections, are seen to contain blood-cells. With this assurance that the heart-valves of the pig contain blood-vessels experiments were begun with the preconceived notion that it would be a simple matter to inject the vessels with carmin-gelatin. Many attempts were made before the proper conditions were found, and forty-six hearts were used before a satisfactory injection was obtained. The important considerations were the post-mortem rigor of the myocardium, the presence of clots of blood or bubbles of air in the small branches of the coronary arteries, the blocking of the large blood channels so that the injected fluid would pass into the valves, and the pressure required to force the gelatin into the small lateral vessels of the annular arteries. When these factors were properly arranged, the blood-vessels of the heart valves of the pig could be injected with regularity.

After these experiments, the procedure was as follows: The heart was used immediately, if obtained within an hour of the death of the animal. If not, it was kept in isotonic salt solution at room temperature for 24 hours, until the rigor of the muscle became soft and the vessels relaxed. The chambers of the heart were washed as free from clot as possible by allowing water to flow through the auricles. The coronary sinus was plugged with cotton and tied off gently. The cut edges of the auricles were then closed with a purse-string suture, and the pulmonary artery was tied off as high above the valves as possible. Small glass cannulae were then passed down the stump of the aorta, into the orifices of the right and left coronary arteries, and tied in place by a silk suture passed with a fine needle around these arteries near the aorta. Care was exercised then to close all open vessels and to cause no new 'leaks' from tissue torn by the sutures. It was found to be of great importance to block these wider channels, so that the injection mass could be forced into the narrower course of the heart-valves. By an arrangement with an air-pressure apparatus, connected with a manometer, the melted carmin-gelatin at a constant temperature of 45°C. was pushed into the coronary vessels under a pressure of 140 to 190 mm. Hg. while the heart was kept in a bath of salt solution at 50°C. This high pressure appeared to be the most important factor in the procedure Injection at the expected coronary blood-pressure of 110 to 120 mm. Hg. did not penetrate into the valves. When, however, the higher pressure was applied, many more small vessels became filled. The injection was held at this stage for half an hour. At the end of this time, while the pressure was maintained at 190 mm. Hg., the warm bath surrounding the heart was replaced with ice-water. When the gelatin had solidified in the coronary arteries, the cannulae were disconnected, and the heart cut open to remove the carmin-gelatin collected in the auricles and ventricles. The opened heart was then placed in 10 per cent formalin to harden the gelatin in the small vessels. After 48 hours or longer, the valves were dissected, removing the atrio-ventricular rings with the tricuspid and mitral valves and strips of the pulmonary artery and aorta with the semilunar valves. Vessels could be seen in the upper portions of the atrio-ventricular valves and sometimes in the chordae tendineae at this stage. In order to render the whole vascular network readily visible, the valves were dehydrated in 95 per cent alcohol, cleared in a mixture of 2 parts of wintergreen and 1 part benzol. In valves rendered transparent in this manner, the vessels could be studied to greatest advantage with the binocular microscope.

In the pig, the atrio-ventricular and semilunar valves and the chordae tendineae are well supplied with blood vessels They differ only in numbers from the vessels of the valves of the human heart and will not be described here in detail.

BLOOD-VESSELS OF THE VALVES OF THE HUMAN HEART

Atrio-ventricular valves

The human hearts used for these injections were carefully selected normal hearts obtained from the autopsies of the Pathological Department of the Johns Hopkins Hospital. Fourteen hearts were injected. These were taken from human subjects of the following ages: 1 to 10 years, 6 hearts; 10 to 20 years, 2 hearts; 20 to 30 years, 2 hearts; 30 to 60 years, 4 hearts. In some of these, only irregular groups of vessels and long delicate arterioles extending to the line of closure of the valves were shown by the injection. In three hearts fairly complete injections were obtained. The following description is based upon these injections:

The tricuspid and mitral valves receive arterioles from the annular branches of the right and left coronary arteries as these pass through the region of the annulus fibrosus at the atrioventricular junction. These small arteries to the valves are not derived from the vessels which supply the auricular musculature, but are distinct branches from the annular arteries passing directly into the valves. The general distribution of these vessels is shown by figure 1, which is a photograph of the mitral valves of a woman, aged 21, who died after an operation for exophthalmic goitre. Autopsy No. 4609. Figure 2 is a drawing of these vessels, made directly from the photograph of the specimen. The chief arteries to the valve branch from the annular artery at widely separated intervals. These points of entrance are almost directly above the papillary muscles, and the vessels pass down the valve in the thickened portion above the insertions of the chordae tendineae. During this downward course, the arteries give off small lateral branches which ramify as delicate arterioles in the upper third of the membranous portion of the valve. When the thickened area of the line of closure of the valve is reached, the arteries undergo multiple branching, forming tufts of vessels in this region (fig. 3). From the terminal arterioles many small vessels anastomose, almost forming glomerular tufts and loops. In this region the capillaries form abundant anastomoses. Only occasional strands of small vessels pass from the line of closure to the free margin of the valves. No vessels were found passing from the valves to the chordae tendinae.

In the upper portions of the valves distinct differences between the vessels may be seen, differentiating arteries from veins. The arteries have irregular edges, with the lumen constricted in some places and expanded in others. A definite constriction occurs wherever the artery branches. The veins, on the other hand, have a smooth contour, and are not so deeply colored by the injection. These features are shown in figure 4, which is a composite drawing made by Mr. Max Brödel, representing in a semidiagrammatic manner the completely injected regions of several specimens.

Chordae tendineae. In the human heart, the vascularity of these structures is slight. The arteries are derived from the

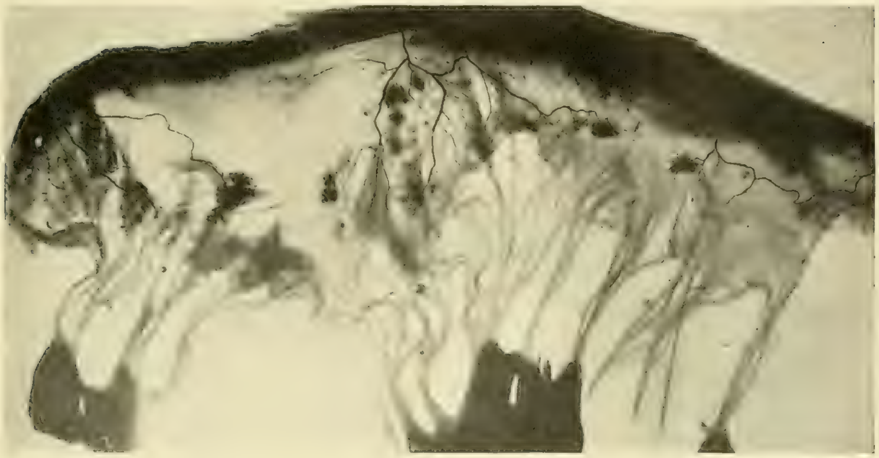

Fig. 1 Photograph of mitral valve, human, showing the blood-vessels injected with carmin gelatin. Enlarged × 3.

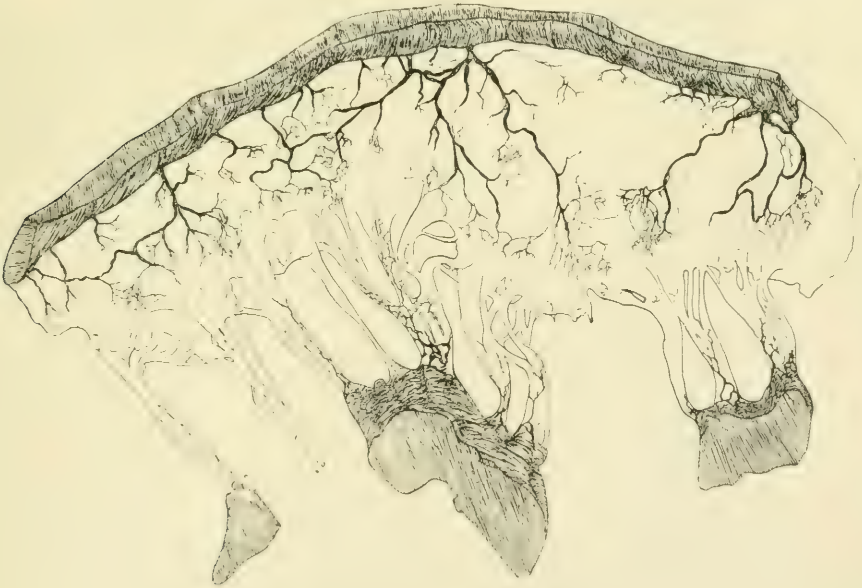

Fig. 2 Drawing made upon the photograph of the human mitral valve shown in figure 1. This drawing exhibits the vascular arrangement more clearly than the photograph. The few vessels in the chordae tendineae are drawn in from another specimen.

branches of the descending rami of the coronary arteries which supply the papillary muscles. These vessels form a subendothelial plexus around the bases of the chordae tendineae, and from this plexus delicate strands of vessels can be seen passing up along the chordae almost to their insertions in the valves.

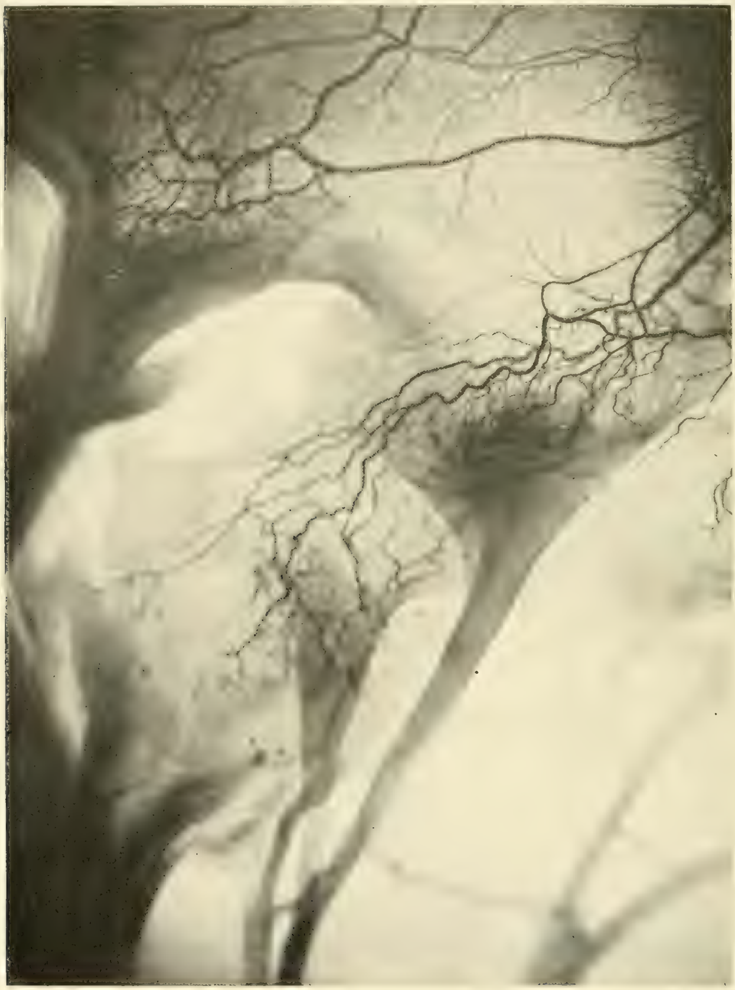

Fig. 3 Enlarged view of the arrangement of the small vessels in the vascular area along the line of closure of the human mitral valve.

Nearly all of these vessels in the chordae lie just beneath the endothelium. Some, however, are situated in the center of the chordae.

The chordae tendineae of the pig's heart have an abundant supply of blood vessels. As these have not been previously described, they are shown here in the following photographs (figs. 5 and 6).

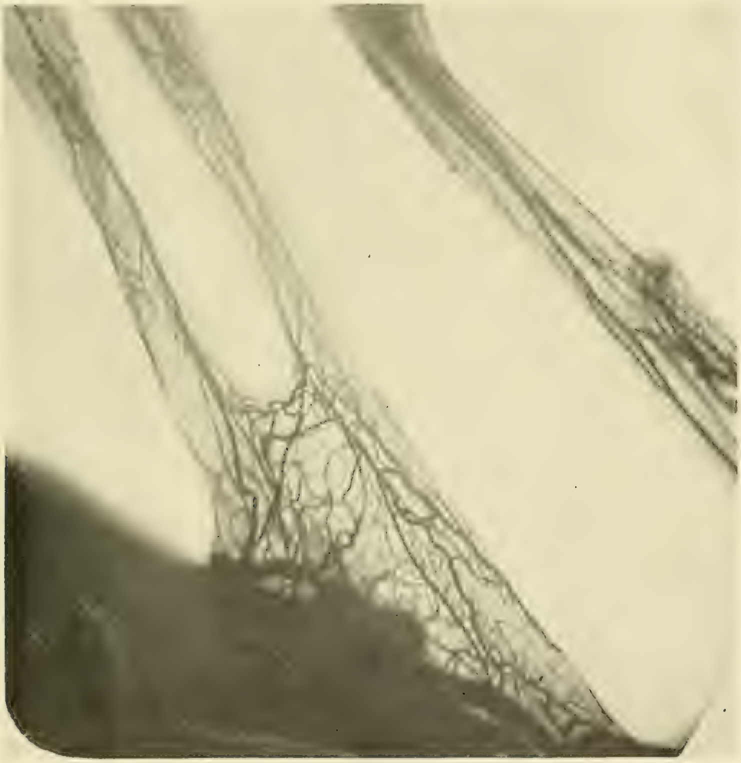

Fig. 4 Photograph of the blood-vessels in the chordae tendineae of the pig's heart, after injection with carmin gelatin. In the human heart, only rudiments of this abundant vascularity are demonstrable.

Semilunar valves

The blood-vessels of the aortic and pulmonary valves arise from two sources, one of which is the vasa vasorum of the arteries, the other the vessels of the auricular endocardium. Injections of the semilunar valves were successful in only three normal hearts. The pulmonic were more easily injected than the aortic, due to the fact that the insertion of the cannulae into the sinuses of Valsalva compresses the blood-vessels of the aortic valves. A drawing made upon a photograph of a specimen of an injected pulmonic valve is shown in figure 7. In this, the few delicate vessels which, arising from the vasa vasorum where the cusps are attached to the wall of the artery, penetrate the

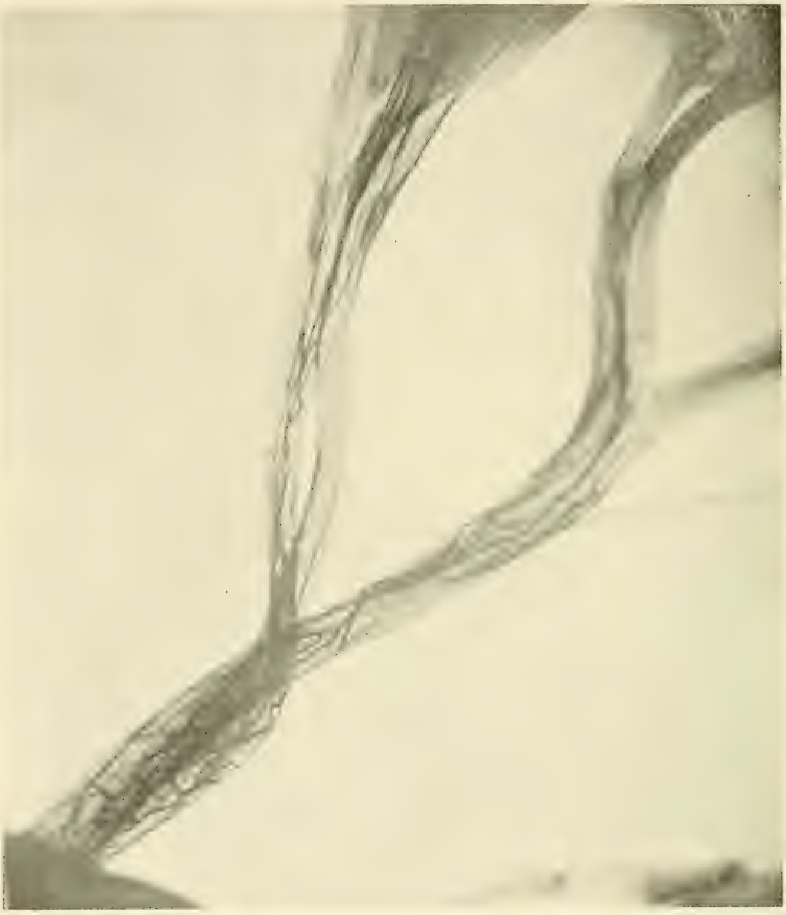

Fig. 5 Injected blood-vessels in the chordae tendineae of the pig's heart.

valve for a short distance along its line of closure are not shown. The vessels going to the valve from the auricular endocardium form a hedge-like plexus in the base of the cusp and from this plexus delicate vessels pass upward for a distance of about a half of the extent of the valve. No vessels were seen in the thin central portion of the valve or in the noduli Arantii.

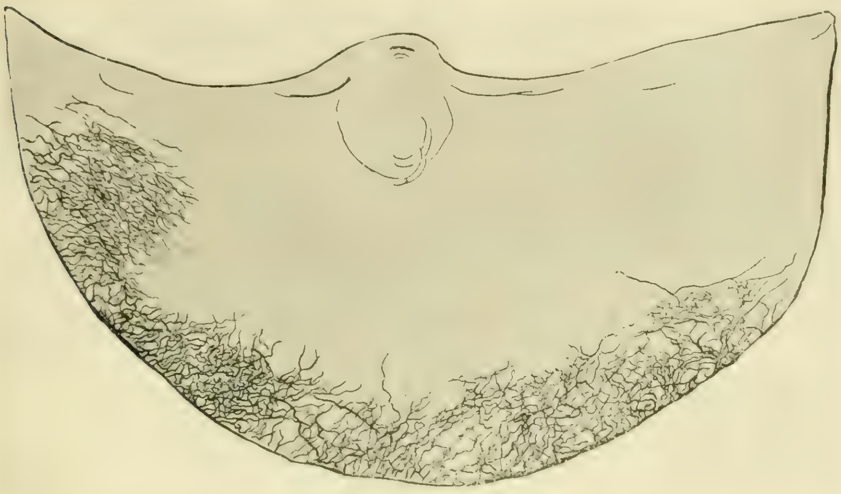

Fig. 6 Drawing made upon the photograph of a specimen of human pulmonic valve injected with carmin gelatin. This shows that vessels penetrate the semilunar valves from all sides and reach almost the center of the valve.

SUMMARY

The studies may be summarized as follows:

1. By the injection of carmin-gelatin under pressure of 160–190 mm. Hg. into the coronary arteries, blood-vessels are demonstrable in the valves of the normal human heart.

2. The atrio-ventricular valves receive distinct branches from the annular divisions of the coronary arteries. These vessels pass into the valves at the thickened regions above the insertion of the chordae tendineae and ramify chiefly along the line of closure of the valves. Arteries, veins and an anastomosing capillary bed are recognizable in these locations.

3. The chordae tendineae receive a few small arterioles from the vessels of the papillary muscles. These traverse the chordae tendineae chiefly beneath the endothelium covering these structures.

4. The semilunar valves receive a definite blood supply from the vasa vasorum of the pulmonary artery and aorta and from the endocardial vessels of the auricles.

5. These vessels afford a definite anatomical basis for the conception that endocarditis may be embolic in its origin.

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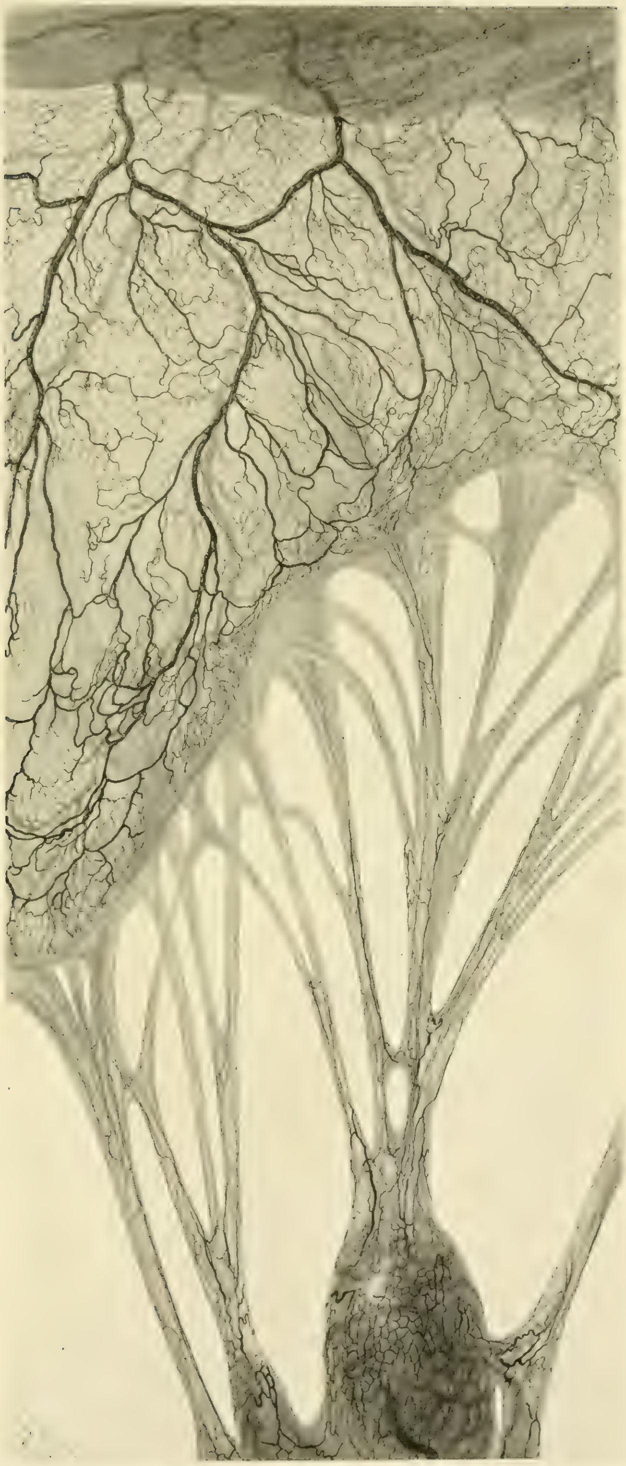
PLATE 1

Semidiagrammatic drawing made by Mr. Max Brödel based upon several specimens, giving a composite picture of the typical vascular anatomy of a completely injected human mitral or tricuspid valve. Enlarged × 6.5.



魚類胎兒細胞內ノ「こんどりおーむ」

ジュールス、ドュエスベルグ 述

魚類ノ胎生時細胞ノ原形質ハ高等有脊椎動物及無脊椎動物ノ胎生細胞ノソレト同一ノ造構ヲ有シ、主トシテ基質及「こんどりおーむ」ノ二物質ヨリ成ルガ如シ。早期ニ於テハ（例ヘバ「ぶらすとみあ」期）「こんどりおーむ」ハ顆粒狀ヲナシ、特ニ邊緣細胞ニ豐富ニ見ラルルモ、後（「ふぁんでゅらす」胎生第二日）其大部ハ絲狀トナル。 此物質ハ魚類胎兒各種臟器ノ凡テノ細胞內ニ存在ス。 本論文ハ上皮、中樞神經系、神經細胞、脊索、中胚葉、血球、ウォルフ氏管、腸、元始生殖細胞及筋胚節內ニ於ケル該物質ヲ精細ニ敍述セルモノナリ。

CHONDRIOSOMES IN THE CELLS OF FISH-EMBRYOS

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EIGHT FIGURES

Since Meves' ('08) discovery of chondriosomes in the cells of the chick-embryo, these cytoplasmic elements have been found in the embryonic cells of other birds, of mammals and of amphibians among the vertebrates, and of insects, of worms and of ascidians among the invertebrates. The only description of similar structures in embryos of the lower vertebrates (i.e., fishes) has been given by Aunap ('13), in his paper "Ueber die Chondriosomen der Gonocyten bei Knochenfischen." Aunap, as the title of his paper indicates, was especially concerned with the study of the primordial germ-cell. His purpose was to find out whether, in Coregonus maraena, as Rubaschkin ('10) and Tschaschin ('10) had described for mammals and birds respectively, the chondriosomes of these cells were granules—a point to which Rubaschkin was inclined to ascribe great theoretical importance. Confirming the above mentioned authors, Aunap found only granules in the primordial germ-cells. In the other cells of the germinative epithelium, however, as well as in the epithelium of the intestine, of the neural tube, and of the Wolffian ducts, filaments were present. In younger stages (i.e., in the blastomeres), Aunap found granules exclusively.

I have had the opportunity of extending these observations by the study of embryos of several species of fishes: the white perch (Morone americana), the yellow perch (Perca americana), the rainbow trout (Salmo irideus) and Fundulus heteroclitus. The first three of these were obtained through the courtesy of the United States Bureau of Fisheries from the hatcheries at Havre-de-Grace, Maryland, and White Sulphur Springs, West Virginia. For Fundulus, I am indebted to the Biological Laboratory of Woods Hole, Massachusetts and I am glad I have this opportunity of expressing to Professor F. R. Lillie my gratitude for his kind hospitality. The results obtained were practically the same for all species; unless otherwise stated, my description will refer to Fundulus. My material consisted of embryos of this fish of various stages from that of nine hours after dry fertilization up to the ninth day. To Professor Stockard I am indebted for a demonstration of this procedure of fertilization. Specimens of all stages, up to hatching, of both kinds of the perch and of the trout were also procured. My series are unfortunately incomplete, for I experienced at first many difficulties in obtaining good preparations; these troubles were overcome only when most of the material was used up.

The fixatives employed were first, the mixture of formol-bichromate after Regaud, secondly, Benda's fluid and several modifications of it.

After treatment with Regaud's fluid, a good number of the embryos of all species mentioned showed excellent preservation of the chondriosomes, but even then, they could hardly be used for an embryological study. This difficulty is proportionally greater in the younger embryos. The outlines of the different organs are usually poorly defined. Some tubular organs (the intestine or the Wolffian ducts, for example) shrink considerably in many specimens. The limits of the cells cannot, for the most part, be distinguished at all, even in those cases in which, after fixation with Benda's fluid, they appear as plainly as they do between the epidermic cells, as represented in figure 2a. The yolk has a great tendency to swell and consequently to dislocate the cell-structure,—a phenomenon which I had already experienced (cf. Duesberg, '15) and which I observed again this summer, in dealing with ascidian-embryos. In these, the swelling of the yolk is especially noticeable in certain stages and in certain cells.

The technical results obtained on these fish-embryos seem to be, within certain limits, independent of the respective duration of action of the formol-bichromate mixture and of the bichromate solution subsequently used, provided the total duration of action be not too short; excellent fixation of the chondriosomes was obtained in one case (lot 38) after six days in formol-bichromate and six days in bichromate, while in another case (lot 39) similar results were obtained after one day in formol-bichromate and eight days in bichromate. The preservation of the material was not affected by leaving the embryos in a cloudy formol-bichromate solution or by changing the solution each day. Although Regaud's fluid is supposed to penetrate better than Benda's, a number of embryos fixed in the former showed the chondriosomes well preserved in the outer layers and very poorly in the chorda. The jelly surrounding the eggs of many fishes (as in the perch) proved to be an insurmountable obstacle to good fixation. Poor preservation of the chondriosomes is indicated, as I have shown ('10, 2), by the breaking of filaments into granules, the swelling of granules and their transformation into vesicles.

Eggs of both kinds of perch and of Fundulus were fixed in the regular Benda's mixture (1 per cent chromic acid, 15 cc.; 2 per cent osmic acid, 4 cc.; acetic acid, 2 drops, in some cases, none at all). Eggs of trout were preserved in the same mixture and also in the following modifications of it:

(1)	0.5 per cent chromic acid + 1 per cent NaCl	15 cc.
	2 per cent osmic acid	4 cc.
	Acetic acid	3 drops

which is the formula proposed by Meves ('08) for the chick-embryo.

(2)	Benda	two parts
	NaCl 1 per cent aqua	one part
(3)	Benda Locke's solution	equal parts
(4)	0.5 per cent chromic acid + 0.5 per cent urea	15 cc.
	2 per cent osmic acid	4 cc.
	Acetic acid	3 drops

the addition of urea being suggested by a recent paper of Allen ('16).

All these mixtures were used with approximately the same results. Chondriosomes are fixed, as is well known, only in the peripheral layers, but the general preservation of the embryo is excellent. The cell-limits are very conspicuous.

Irrespective of the fixative employed, one may notice in Fundulus embryos as young as two days, that occasionally and quite independently of the penetration of the fixative, the chondriosomes in the different tissues are not equally well preserved. I have repeatedly found, for instance, that the chondriosomes of the intestinal epithelium, or of the blood-corpuscles, were well fixed, when no chondriosomes at all could be seen in the neighboring cells. I should consider this an indication that, already in these early stages, a differentiation of the chondriosomal substance has taken place in the different tissues. It was found furthermore that some difference in the chondriosomes exists between the posterior and the anterior part of an embryo (as also between the regenerated and the regenerating part of the tail of a triton), as it very often appeared necessary to carry the differentiation of the stain to different degrees depending on the part desired for study.

Besides a few trials on Fundulus with a vital dye, Janusgreen, which gave successful stains only in the epidermic cells (these experiments were, however, not carried very far), three stains were resorted to,—iron-haematoxylin, Benda's sulfalizarin-crystall-violett and acid fuchsin-methylgreen. The first named gives occasionally very good preparations. The second one gave no useful preparations whatever when applied after Regaud's fixation, and only very few good ones (two or three on some 150 embryos) with the material fixed in any of the chromosmic mixtures mentioned above. A similar experience was met with when it was attempted to stain the testicle of a fish, after fixation in Benda's fluid. These technical difficulties were not due to the staining reagents, for the same set, applied to a familiar object (the mammalian testicle) worked perfectly; it was finally discovered that nothing was wrong with the material either, for, to my great surprise, acid fuchsin-methylgreen gave excellent preparations. From this, two conclusions may be drawn, interesting enough, in my opinion, to justify this narration of my experience: the first one is that on a given material, one chondriosomal stain may give satisfactory preparations when the other does not; and the second one, still however hypothetic, is that fish-tissue is, for some reason, refractory to Benda's stain.

The fuchsin-methylgreen stain was used according to Cowdry's description ('16, 2), except that the use of a 10 per cent solution of fuchsin in anilin-water proved as satisfactory as the usual 20 per cent solution. Furthermore, I stained in a flat-bottomed evaporating dish, instead of staining on the slide. My way of proceeding is cleaner and more economical than the other way, and, what is more important, this method gives a perfectly regular stain all over the slide.

After either fixation, I was unable to cut sections 5 μ thick through the whole embryo. The yolk-sac had to be removed—an easy procedure during the first twenty-four hours and after the third day, but a rather difficult one during the intermediate period.

I shall continue to use the nomenclature to which I came back in my last paper ('15): mitochondria (granules), chondrioconts (filaments), chondriosomes (general term). This nomenclature is based exclusively on morphology; it does not lead to any confusion with other terms and it is already the most widely adopted.

In a review recently published, Cowdry ('16, 1) advocates the use of the term 'mitochondria' in a general sense.

> American investigators, writes Cowdry (p. 424), have with few exceptions from the beginning employed the term 'mitochondria,' exclusively, recognizing well that the same material, under different conditions, may assume special forms. Even the word 'mitochondria' leaves many things to be desired, but it is in general use, it is descriptive of morphology only and it does not commit the user to any hypothesis of the functional significance of the material in question. True, we cannot use the name in the exact sense that Benda, who introduced it, used it, any more than we can employ the term cell with anything like its original meaning. Yet no one would invite us to give up the word cell and to substitute a new and more appropriate term in its place.

To this, I take the liberty of making the following remarks. The term 'mitochondria,' in Cowdry's sense, is not in general use even in this country; if the nomenclature must be based on morphology, one should, to be logical, reject 'mitochondria' as a general term, for 'mitochondrium' means granule and cannot in consequence mean filament. The analogy with the term 'cell' is not adequate, for, though this word is not appropriate, it is, however, generally adopted; and it should not be forgotten that the invitation to use a term in a different sense than its original meaning comes in this case precisely from those who want to use 'mitochondria' as a general expression.

Confusion in the cytological nomenclature is due mainly to two causes. One of these causes is that the same things have often been described under different names, without the authors being aware that they were dealing with the same structures; recent papers, especially those of Meves, have cleared, or should have cleared the situation very much, if it were not for the fact that they are too often ignored or misunderstood, (we know, for example, that many of the things which have been described under the name of plastidules, Nebenkern, bioblasts, microsomes, etc., are chondriosomes). Another cause of confusion in the nomenclature has been the creation of new terms, under which heading falls the use of the expression 'mitochondria' in a general sense. This, however, is a defect of human nature, not a defect special to the cytologist, and against which we are helpless. As an example of the overcrowding of every field in anatomy with synonyms, I simply refer to a recent article by Rabl ('16), in which the nomenclature used in the description of the first stages of the development of the embryonic shield of birds and mammals is exposed.

My observations made on the blastomeres of Fundulus (nine hours after fertilization) agree with those of Aunap on Coregonus: mitochondria exclusively are found in this stage. The granules are scattered throughout the cell-body, but are especially accumulated along the cell-limits. No change in shape is observed during mitosis, nor is there any special disposition in the dividing cell, other than the usual accumulation between the daughter-nuclei during the ana- and telophases. The marginal cells deserve special mention, for they are, as well as is the periblast, remarkably rich in mitochondria. This fact accounts undoubtedly for the special coloration of these cells,—a phenomenon which students of the segmentation of the fish-egg have mentioned; Agassiz and Whitman ('84) have already described, in Ctenolabrus, differences between the staining properties of the central and the marginal cells, and later Kopsch ('01) expressly mentioned a "dunklere Färbung der Randsegmente" in Belone acus.

Figure 1 represents a cross-section (the 37th,—5 μ thick—from the posterior extremity) of an embryo of Fundulus, fixed in Regaud (lot 38) and stained with acid fuchsin-methylgreen. The embryos of this lot were fixed three days after fertilization but are perhaps, judging by the rather scarce data I could find in the literature, somewhat more advanced than the average; I counted in them 28–30 somites and the Kupffer's vesicle had disappeared.[1] Figure 1 shows the epiderm, the nervous system, the somites, the chorda, a blood-vessel with a few blood-corpuscles and the periblastic layer (unfortunately broken in one place) with its nuclei and two pigment-cells. A very marked change has taken place in the structure of the cells. Few mitochondria and more numerous chondrioconts are found now, and the same holds true throughout the whole embryo, the end-bud included. On the whole, from the cytological standpoint, such a preparation resembles very closely similar preparations of chick-embryos of about the same age; there are perhaps (but this is a detail of minor importance) a larger number of loops and circles in the fish-embryo. These circles are not to be confused with the vesicles found in poorly fixed objects. Owing to the lack of material, I cannot tell how this change took place; I am unable to decide whether these rods were formed by mere elongation of the mitochondria observed in the blastomeres, or by fusion of these granules, as I have found in the rabbit-embryo ('10, 1).

I come now to a somewhat more detailed description of the chondriosomes in the different tissues.

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[1] Shrinkage due to the action of Regaud's fluid might however, be responsible for this.

The epidermic cells of the superficial layer (fig. 2a) contain a large number of chondriosomes, most of which are long, curved filaments, exhibiting a distinct tendency to run parallel to each other, or the surface of the cell or of the nucleus. Such a disposition is of course especially conspicuous in a surface-view of the epiderm. Chondriosomes are not so abundant in the cells of the deeper layer. Quite remarkable are some large cells which I suppose to be the glandular cells of the epiderm, though I never saw any indication of a secretion in them. These cells have already appeared during the second day, in Fundulus, and apparently differentiate from cells of the deeper layer. Up to the ninth day, there are found almost exclusively in the abdominal wall, near the point of reflection of the epiderm on the yolk-sac, and likewise on the yolk-sac itself in the same neighborhood. The groundmass of the protoplasm hardly stains and includes within it an enormous number of long chondrioconts. This appearance I cannot help comparing to that of a bunch of hair, tied together at the top of the cell and then spreading out through the whole cell-body. The nucleus floats somewhere in the deeper or middle part of the cell between the chondrioconts (fig. 2b).

Chondriosomes are also present in the cells of the lens.

In the central nervous system, each cell contains a few long filaments, running parallel to its long axis, and in addition to these, there occur shorter elements, usually in the shape of loops or circles, at the poles of the nucleus (fig. 1). As in many epithelia, the basal part of the cells which reach the basement-membrane contains usually an accumulation of chondriosomes; this accumulation is visible with low power and defines sharply the outlines of the organ. In ganglion-cells, the chondriosomes, as in the chick-embryo (cf. Duesberg, '10, 2, fig. 6) are short and thin threads, located at one pole of the nucleus.

The cells of the chorda in young embryos have long chondrioconts (fig. 1). Later, I find granules, but I am unable to ascertain whether this deeply situated organ was well fixed.

In mesenchyme-cells, chondrioconts are predominant (granules represent most probably the cross-section of filaments).

These filaments are of various lengths and are either located close to the nucleus or extending into the processes (figs. 5, 6c and 7a). In Fundulus, the mesenchyme-cells surrounding the anterior part of the digestive tract (i.e., those situated close to the dorsal wall of the pharynx and in the branchial arches) become, during the fourth day, loaded with large granules. These granules stain intensively with acid fuchsin, crystall-violett and iron-haematoxylin. The same cells contain also long chondrioconts (fig. 3). Altogether, they build up a very dense layer of tissue.

Blood-corpuscles contain a few long filaments (figs. 1 and 4a), often sharply curved, and usually many shorter ones, in the shape of loops or of small circles, accumulated in one or two heaps. As soon as haemoglobin is formed in the red corpuscles, no chondriosomes are visible—a peculiarity I had noted before in the chick-embryo ('10, 2). No attempt, however, was made with the modification of Benda's method, which Shipley ('15) has devised for the special purpose of staining chondriosomes in the red corpuscles.

Besides ordinary leucocytes, the body-cavities and the tissue-clefts in Fundulus hold a peculiar kind of large wandering-cell,[2] in which the chondriosomes are represented by very thin threads, mostly accumulated near the nucleus in dense masses (fig. 4c).

The cells of the Wolffian ducts show at their basis an accumulation of chondriosomes: rods, loops and circles. Long chondrioconts emerge from this accumulation and reach the opposite pole, where some can be seen to bend and run backward toward the basis of the cell (fig. 5). Chondriosomes have already been described in the kidney-cells of the adult fish by Policard and Mawas ('06, '09) and by Regaud ('08).

In Fundulus embryos three days old, I found a striking difference between the disposition of the chondriosomes in the cells of the anterior and of the posterior part of the digestive tube. In the posterior part (fig. 6a), numerous chondriosomes are accumulated below the nucleus, which is, so to speak, imbedded in them. Each cell contains, in addition to these, a few long chondriconts which could actually in some cases be followed from the basal accumulation to the top of the cell, and from there back to the basis. In the anterior part (fig. 6b), the cells are crowded with long, sinuous and rather thin threads, which, followed from the inner pole, are seen to circumscribe the nucleus and intertwine with each other in the basis of the cell.

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[2] Whether these cells have any relation to the four kinds of wandering cells described by Stockard ('15) in the yolk-sac of Fundulus seems rather doubtful.

Later, the cells of the branchial region of the digestive tract become flattened, covering with a thin sheet the dense layer of peculiar mesenchyme-cells described above, and the chondrioconts of these flattened cells run parallel to the surface (fig. 3). Further posteriorly (fig. 6c), the disposition of the chondriosomes is very similar to the arrangement in the cells of the anterior part in the preceding stage, but the cells themselves have grown and differentiated a border. There is an accumulation of chondriosomes below the nucleus and long chondriocohts are found running through the whole cell-body; some of these can, by careful focussing, be followed from one end to the other of the cell. Whilst a number of these chondriocoнts are perfectly smooth, others bear swellings, sometimes a regular row of them. Besides, the cells contain vacuoles, the content of which could not be stained by the methods used, and granules of various sizes, stained red in acid fuchsin preparations. Such an intestine is obviously engaged in an active process of absorption.

Figure 6d represents part of the intestinal wall of an embryo-trout, twenty-four days old. In each intestinal cell, one finds two accumulations of chondriosomes, one above, the other below the nucleus. Between these masses one may make out long chondriocónts which, in some of the cells, bear swellings. Such a disposition recalls exactly Champy's description ('11) of the chondriosomes in the intestinal cell of higher vertebrates. In the adult fish, they have been described by Corti ('13) who, in Box salpa and in Trinca vulgaris, found a few short chondriocónts and an accumulation of chondriosomes in the distal part of the cell of the fasting animal, whilst, during absorption, vacuoles appear in the cytoplasm and the chondriocónts seem to fall to pieces. I cannot help saying that the scarcity of chondriosomes and of chondrioconts especially, which Corti represents in his drawings, suggests to me imperfect preservation. Judging by Aunap's figure 4, it appears that the disposition of the chondriosomes in the intestinal cells of the embryo of Coregonus is quite different from what I found both in Fundulus and in trout.

I have also had the opportunity of making a few observations in connection with the question of the primordial germ-cells.

In embryos of Fundulus of three days and older, I found a number of cells, the large size of which attracted my attention. At nine days, the last stage I have studied, such cells can be made out anywhere in the embryo (some are identified between peritoneum and intestinal wall), but most of them are located in the anterior region of the trunk, in the mesenchyme which occupies the space comprised between pharynx and central nervous system. Felix and Bühler ('06) state that the primordial germ-cells of the teleosts are remarkable because of their large size and the lightness of their cytoplasm and nucleus. Aunap ('13) (see also Dodds, '10) expressly mentions that these cells do not contain any special yolk-granules nor centroteka, in contrast to the primordial germ-cells of birds. The cells I find in Fundulus have these characteristics and furthermore, no other cells were seen which could be considered primordial germ-cells. Against their qualification as such, I must, however, mention their close connection with the surrounding mesenchyme-cells (fig. 7a) or with endothelial cells. This latter connection is so frequent that I at first thought these large cells were merely endothelial cells seen in a tangential section of a vessel,—an idea which for several reasons I was finally induced to drop. Such connections have never been mentioned before, but on the contrary, the majority of authors expressly state that the primordial germ-cells are round or oval, and not stellate, in shape. I am unfortunately unable to decide as to the significance of these cells, as, at nine days, no genital gland is yet developed in the embryo of Fundulus.

Another point of variation is the difference between the chondriosomes of these cells and those described by Aunap in the primordial germ-cells of Coregonus. Aunap found only granules; in Fundulus, these cells contain filaments, most of them in little heaps and in close relation to the nucleus. Supposing that these cells are primordial germ-cells and that consequently a comparison with Aunap's results is justified, such a difference may be explained in several ways: by admitting either the existence of specific variations, or a change in the shape of the chondriosomes with the evolution of the germ-cell, or, finally, the imperfect preservation, in Aunap's preparations, of these deeply situated parts of the body of the embryo. It must, however, be added immediately that the question of the shape of the chondriosomes in the primordial germ-cells has completely lost its importance, since Swift ('14) has demonstrated, contradicting Tschaschin, that in birds these chondriosomes "are not at all characteristic; they resemble the mitochondria of the somatic cells (p. 495)."

Curiously enough, if my findings on these cells of Fundulus agree with Felix's description of the primordial germ-cells of the Salmonides, my own observations on Salmonides do not agree with that description of Felix. In the rainbow trout, the primordial germ-cells, easily recognized as such in the genital region, are conspicuous, not only because of the size of the cell and of the nucleus, but also by the presence of peculiar globular inclusions. I found in these cells three kinds of bodies: 1) fat-droplets, nearly always conglomerated in one large globule and located at one pole of the nucleus; 2) granules scattered throughout the whole cell; these stain lightly in red in acid fuchsin-methylgreen preparations, in pink with Benda's stain; 3) very minute mitochondria (fig. 7b). The fat-globule is dissolved after fixation in Regaud and none of these three kinds of bodies is preserved in Zenker; this explains perhaps why Felix did not see them. In this case, my observations on chondriosomes agree with Aunap's, except in that the granules he figures are larger. Whether the granular form corresponds actually to the form of the chondriosomes in the living cell is hard to decide, for the genital region is not easily reached by the fixing fluid.

Figures 8a and 8b represent sagittal sections of two myotomes from the same embryo. In both, the dorsal edge is formed by cells multiplying actively and moving ventrally to differentiate into myoblasts. A frontal section would show the muscle-plate and the cutis-plate; the latter, in fishes, persists much longer than in birds and mammals. The constitution of the muscle-plate is difficult to elucidate. For birds and mammals, however, I am of those who believe that the myoblasts are well individualized cells; I found ('10, 2) that the distribution of their nuclei does not agree with the syncytial theory and I never failed to see the cell-limits on cross-sections of embryos fixed with Flemming's fluid or a modification of it (Duesberg, '10, 2, figs. 17 and 19). I want to emphasize this viewpoint as opposed to that of Huber ('16), who is inclined to believe that, in the rabbit, "the syncytial character of the cells from which the voluntary muscle is developing is evident" (p. 168). The reasons why Huber failed to see the limits of the myoblasts are in my opinion obvious: he used longitudinal sections, when only cross-sections can give the clue, and he made the sections too thin (2 and 3 μ thick), for, the thinner the sections, the less conspicuous will the cell-limits appear. I desire to call attention also to what I wrote on page 466 about the action of certain fixatives on the cell-limits.

I come now to a consideration of the chondriosomes of the cells of the somites. In the very young myotomes (i.e., before any differentiation of the inner layer has taken place) and later in the cells of the dorsal edge and of the cutis-plate, the chondriosomes are for the most part long filaments, which may entirely circumscribe the nucleus. Figures 8a and 8b show such a disposition very well in the cells of the dorsal edge.

In the myoblasts, there occur very marked differences in the pictures of these cytoplasmic constituents in the stage before as compared to that after the development of myofibrils. In the first case (fig. 8a), the myoblasts contain very numerous chondriosomes at both ends and also a large number of these bodies in the intermediate portion of the cell; these intermediate elements are either very long chondrioconts extending to both ends of the myotome, or shorter filaments disposed in longitudinal rows. After the appearance of the striated myofibrils, which in embryos fixed with Regaud's fluid are, as fig. 8b shows, very difficult to see, nearly all the intermediate chondriosomes have disappeared and only the accumulations at both ends of the myoblasts persist.

I do not intend in this paper, to dwell on the topic of the rôle of chondriosomes in histogenesis. I am preparing an extensive study of it, both in normal development and in regeneration. I can, however, state now, that all I have seen corroborates the conviction I gained from the study of the chick-embryo ('10, 2); and such preparations from the fish-embryo as I just described, without being conclusive, are certainly in favor of my opinion. Hence, a discussion of the bibliography more naturally belongs to this next study; I take, however, the liberty of making the following remark about a recent paper by M. R. Lewis ('17)[3]. M. R. Lewis found, that in tissue-cultures of connective-tissue cells, there appear fibers with the differentiation of which chondriosomes have seemingly nothing to do. To draw, however, from such observations any conclusions extending to the normally developing embryonic tissue, in which the nature of the differentiations are beyond doubt, seems to me hardly legitimate. The results which Baitsell ('15) obtained, show how careful we ought to be in our interpretations.

I come now to a consideration of the behavior of the chondriosomes in dividing cells.

In his first paper on the chondriosomes of the chick-embryo, Meves ('08) came to the conclusion that, in contradistinction with what frequently appears to be the case in the spermatocytes of invertebrates,

> der Ablauf einer Mitose scheint auf das Verhalten und die Lagerung der Mitochondrien und Chondriokonten gänzlich ohne Einfluss zu sein. Die Mitochondrien erhalten sich während der Teilung als solche und nehmen keine besondere Anordnung an; ebenso bleiben auch die Chondriokonten unregelmässig durch den Zellleib verteilt (p. 840).

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[3] Which I was enabled to read in the proofs, through the courtesy of Mrs. M. R. Lewis.

Both Meves ('10, 1) and I ('10, 2), independently, reported later that certain changes may occur in the chondriosomes during the division of the embryonic cell of the chick, especially in later stages than those first studied by Meves. The chondriosomes are very often found, during the ana- and telophases, accumulated between the daughter-nuclei, whilst their shape, size and number appear to have changed during the mitotic process (cf. Meves, p. 154 and Duesberg, p. 616–617). Such modifications exist also in the dividing cells of the fish-embryo. The accumulation of chondriosomes between the daughter-nuclei has been mentioned above, in connection with the description of the blastomeres of Fundulus, and the phenomenon is well illustrated by the dividing blood-corpuscles represented in figure 4b. The same figure shows also the longer filaments contained in the blood-corpuscles broken to pieces during mitosis. Both processes result obviously, as Meves and I (loc. cit.) have indicated for the chick, in an apparently equal repartition of the chondriosomal substance between the daughter-cells.

Concerning their studies of the chondriosomes in the dividing cells of the chick-embryo in tissue-cultures, M. R. and W. H. Lewis ('15) write:

> Many observers believe that the mitochondria form a palisade about the spindle during late anaphase and then divide and one-half of each mitochondrium passes to each daughter-cell (Benda, Meves, Duesberg, etc.) (p. 365). . . . . . A study of the fixed specimens seems to show that the mitochondria retain somewhat their original character and shape during mitosis. They are however almost always shorter and more scattered through the cytoplasm than in surrounding cells. . . . . There is no indication in the fixed specimens of any arrangement of the mitochondria about the spindle in such a manner that they would undergo division into two parts in the plane of cleavage of the dividing cell. On the other hand, all of our specimens seem to show that the mitochondria tend to become more evenly scattered through the cytoplasm during division, and those that happen to be on either side of the cleavage plane are carried into the respective daughter-cells (p. 366).

The same description is repeated on page 371 in connection with the living cell and the authors add: "A division of mitochondria such as observed by Meves ('08) and Duesberg ('10) was never observed. We find as did Buchner that this characteristic arrangement of the mitochondria during division of the cell is by no means a constant occurrence."

Disregarding the fact that Buchner is no authority whatever on these matters and that in his first paper ('08) Meves does not mention any division of chondriosomes, I want to emphasize the fact that I never have considered the division of chondriosomes as a general process. M. R. and W. H. Lewis, however, were obviously under that impression, though their very observations on the behavior of the chondriosomes during mitosis in tissue-cultures of the chick-embryo agree very well with mine on fixed preparations of the same material. In fact, I have described all possible processes in the dividing cell, the observations being made on various objects. That the chondriosomes may dispose themselves in a palisade of rods around the spindle, to be separated into equal parts during the division of the cell, is evident in the spermatocytes of many invertebrates and especially in Blaps (cf. Benda, '99 and Duesberg, '10, 3). In the spermatogonia of Triton, however, I observed a very different process, inasmuch as some of the chondrioconts were seen to pass undivided to one daughter-cell ('10, 3, fig. 46), exactly as I had already figured the phenomenon for the chick-embryo ('10, 2, fig. 8). Finally, in Ciona, still another process takes place,—a constantly unequal repartition of the number of chondriosomes between the daughter-cells.[4]

Summarizing our knowledge of the behavior of the chondriosomes during mitosis, I come to the conclusion that cells may be divided into two categories: those in which the chondriosomes are equally, and those in which the chondriosomes are unequally divided between the daughter-cells. To the first category belong apparently some somatic cells and obviously the male germ-cells. The equal repartition of the chondriosomes is obtained in one of three ways: first, by the formation of rods, or rings, and their bipartition (a most interesting case has been recently described by Wilson—1916—in the Arizona-scorpion); secondly, by the scattering of the chondriosomes through the body of the dividing cell, and, finally, by the attribution of an equal number of bodies of the same shape and size to each daughter-cell (Sokolov, '13). In the male germ-cell, any one of these processes may lead, as I pointed out in 1907, to a reduction of the quantity of chondriosomal substance in the spermatids, an opinion which the observations of Sokolov and of Wilson—on the Arizona-scorpion—clearly corroborate.

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[4] Relating her observations on the normally fertilized egg of the sea-urchin (Strongylocentrotus lividus), Danchakoff ('16) writes (p. 583): "The sector of the radiation known as the spindle seems to be formed by the substance of the plastosomes (M. Lewis, Robertson), or archoplasm (McClung), and consists of uniform thin threads." To this, I take the liberty of objecting on the following grounds: 1) that Lewis and Robertson ('16) do not mistake the chondriosomes for the spindle; 2) that the relationship between the spindle and the chondriosomes has been established by Benda, as early as 1899; the long chondrioconts he found in Blaps surround the spindle, sometimes very closely, but do not form its substance; 3) that, in the egg of the sea-urchin, the chondriosomes are not filaments, but granules (Meves, '12).

A constantly unequal repartition of chondriosomes during mitosis is especially obvious in the ascidian-embryo. Such a behavior is, in my opinion, just as interesting as the phenomenon of the equal repartititon: it illustrates beautifully the importance of the division of the protoplasm. There is a suggestion that an analogous process takes place in certain male germ-cells, as, for example, in Myxinoids (A. and K. E. Schreiner, '08: see however, the reservations I made, '12, p. 683), or perhaps in the California-scorpion (Wilson, '16). If this were confirmed, I could only recall that I have already propounded the question ('12, p. 683) as to whether such a constantly unequal repartition of chondriosomes between the spermatids may not lead to the formation of two kinds of spermatozoa of different physiological value.

I will conclude this paper with a few considerations on the structure of the protoplasm.

From his observations on the centrifuged eggs of Crepidula, Conklin ('17), comes to the interesting conclusion that the ground-substance of the protoplasm, i.e., the protoplasm minus "microsomes, mitochondria, as well as yolk, oil and other inclusions," is formed of two parts, one more fluid part (enchylemma, alveolar substance, paramitome, hyaloplasm, trophoplasm) and one more solid part (reticulum, interalveolar substance, mitome, spongioplasm, kinoplasm). This second part, viscid and elastic, may be stretched and distorted by centrifuging, but it is capable of recovering its normal form afterward. It forms a framework running through the cell and connecting the nucleus and centrosome or centrosphere, with a peripheral layer surrounding the entire egg. This framework is the seat of the polarity and pattern of organization of the cell; it holds the cell-organs, especially the centrosphere and the nucleus, in a definite relation to each other and to the cell-axis—it prevents the complete stratification of cell-substances into sharply marked zones according to their specific weights. The substance of this framework is probably identical with the 'ground-substance' of Lillie, though in Crepidula it constitutes a relatively small part of the cell-contents and, in Conklin's opinion, does have "a filar, reticular or alveolar structure."

The chief and real interest of Conklin's observations lies, in my opinion, in the fact that they might afford an explanation of the behavior of centrifuged eggs in further development. They show the existence in the ground-substance of the egg, of a special, firm architecture, which determines, or helps to determine the polarity.[5] Whether, however, from these observations on the egg-cell, general conclusions may be drawn, is another question: the egg is not of a simple, but of a complicated cytoplasmic structure. It must not be inferred that I intend to deny the existence of zones of different consistence, of a special repartition of gels and sols in other cells; their existence can be deduced from the study of fixed material and is corroborated by the experiments of microdissection. But the same experiments show that the consistence of the different parts of the cell-body changes during the life of the cell, and the study of fixed material teaches that a centrosphere, for instance, may not exist, as such, in a young cell, and yet may differentiate later,—a view which is supported by the occasional incorporation within its substance, of other cell constituents, like chondrioconts.

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[5] Concerning connections between asters and egg-periphery, see Van Beneden and Neyt ('87).

With the recognition that zones of different consistence may exist in a cell, the question of the structure of protoplasm is far from being exhausted. My view of the problem is the following. Protoplasm appears to me to be composed essentially of two substances, a ground-substance and the substance of the chondriosomes. The ground-substance may show, as stated above and emphasized by Conklin's experiments, zones of different density; one of these is the centrosphere. It may furthermore include within it fat or oil, yolk, etc., and, under special experimental conditions, harbour some of the vital dyes. A special structure may develop in the ground-substance, in connection with the centrosphere, mainly during mitosis. Whether, however, it has any other structure, such as a reticulum or an alveolar system, as conceived by Butschli, seems to me to be extremely doubtful, notwithstanding the appearances in fixed preparations. My opinion is based mainly on the movements of the cell-constituents, and especially of the chondriosomes,—movements, the occurrence of which may be safely deduced from the study of fixed preparations and which are demonstrated by the direct observation of the living cell. I agree in this connection with Lillie ('06), whose microsomes are, in part at least, mitochondria, and with M. R. and W. H. Lewis ('15). One might object that the movements described by these latter are exaggerated by the special conditions to which the cells are subjected in tissue-cultures; but it suffices that such movements are possible. One should furthermore be reminded, in connection with the question of the structure of the ground-substance, of A. Fischer's experiments and of the criticism to which Butschli's alveolar theory has been submitted from the standpoint of physico-chemistry, namely by Pauli ('02).[6]

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[6] From this, one will easily understand why I cannot consider as adequate the term 'spongioplasm,' with which Conklin designates the more solid part of the ground-substance. Concerning especially the comparison he makes between his spongioplasm, and, on the other hand, Flemming's mitome and, to a certain extent, Boveri's archoplasm, I recall that it has been demonstrated that part of Flemming's mitome (Meves, '10, 2) and Boveri's archoplasmic granules in the egg of Ascaris (Meves, '14) are chondriosomes.

Of the bodies incorporated in the ground-substance, only the chondriosomes, in addition to the centrioles, can be and should be considered of general significance. Chondriosomes are in fact constant and normal constituents of the protoplasm and it is precisely the result, quite expected, by the way, of the present investigations, to show that the structure described in other groups of animals exists also in fishes, and to allow another step toward the demonstration of its ubiquity. The forms of the chondriosomes are various and changeable, though hardly to the extent that one might perhaps be led to believe from the observations of M. R. and W. H. Lewis ('15) on cells in tissue-cultures. To the student of the male germ-cells, for instance, it becomes obvious that these changes in the chondriosomes are dependent upon certain definite factors and proceed in a definite direction. Of the chemistry of the chondriosomes we begin to have some knowledge: I refer the reader to the valuable summary of that question given by Cowdry ('16, 2) in a recent article. Finally, I am aware that my views in regard to the significance of chondriosomes are far from being generally accepted, but I fail to see how anybody could deny the importance of these cytoplasmic constituents. The recent tendency to investigate the problem from the experimental side should, in my opinion, prove very valuable in helping to solve some important aspects of the subject.

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PLATES

PLATE I

EXPLANATION OF FIGURES

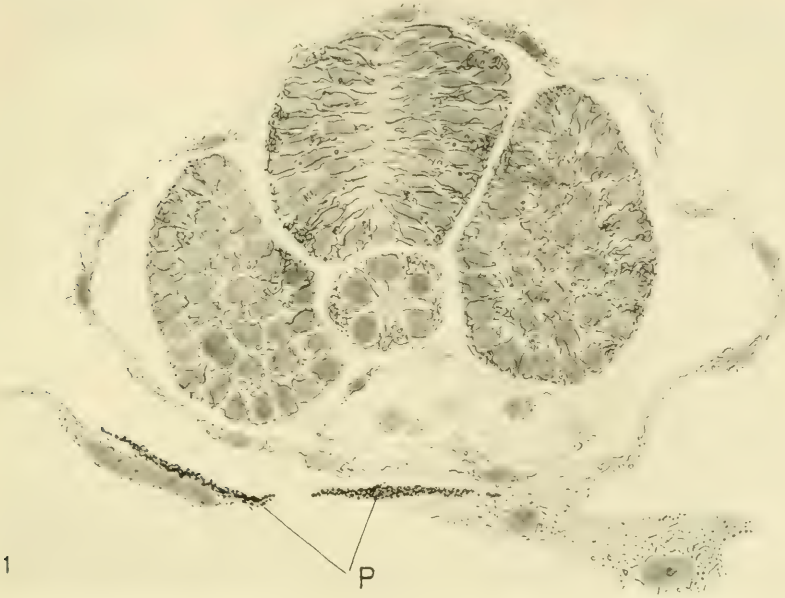
All figures were outlined with a Zeiss camera-lucida, at the level of the stage of the microscope. Lens used: Zeiss apochromatic immersion 2 mm., 1, 40. Artificial light (gas).

1 Transverse section of an embryo of Fundulus, three days old. Fixation: Regaud; stain: fuchsin-methylgreen. Ocular 4. *P*, pigment cells.

2a Surface view of an epidermic cell (upper layer) of an embryo of Fundulus, nine days old. Fixation: Benda (without acetic acid); stain: fuchsin-methylgreen. Ocular 8.

2b Epiderm of an embryo of Fundulus, nine days old: perpendicular section. Two layers of cells and glandular cell. Fixation: Regaud; stain: fuchsin-methylgreen. Ocular 12.

3 Section perpendicular to the epithelium of the branchial region in an embryo of Fundulus, nine days old. Pharyngeal epithelium and peculiar mesenchyme-cells. Fixation: Regaud; stain: fuchsin-methylgreen. Ocular 12.



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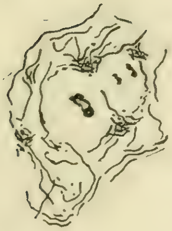

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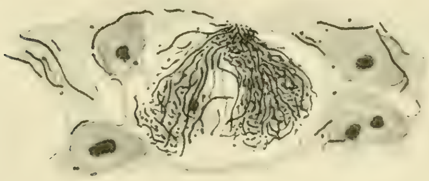

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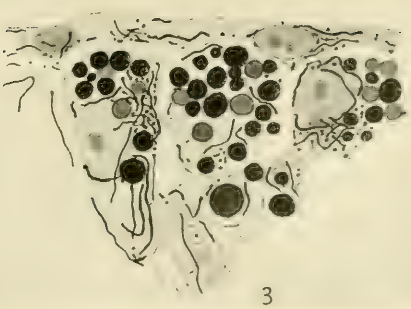

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J. Duesberg, del.

PLATE 2

EXPLANATION OF FIGURES

4a Blood corpuscle, embryo of Fundulus three days old. Fixation: Regaud; stain: fuchsin-methylgreen. Ocular 12.

4b From the same embryo: anaphase of the mitosis of a blood corpuscle. Ocular 12.

4c Wandering cell from peritoneal cavity, embryo of Fundulus, nine days old. Fixation: Benda (without acetic acid); stain: fuchsin-methylgreen. Ocular 8.

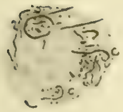
5 Cross section of a Wolffian duct, with neighboring mesenchyme-cells, of an embryo of Fundulus, three days old. Fixation: Regaud; stain: fuchsin-methylgreen. Ocular 8.

6a Posterior part of the intestine of an embryo of Fundulus, three days old. Fixation: Regaud; stain: fuchsin-methylgreen. Ocular 12.

6b Anterior part of the intestine of the same embryo. Ocular 12.

6c Intestine of an embryo of Fundulus, nine days old. Fixation: Regaud; stain: fuchsin-methylgreen. Ocular 12.

6d Intestine of an embryo of Trout, 24 days old (the other embryos of the same lot hatched two days later). Fixation: Benda; stain: Benda. Ocular 12.



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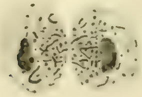

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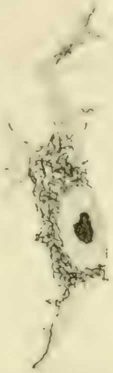

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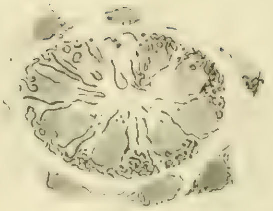

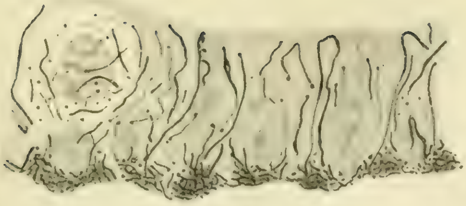

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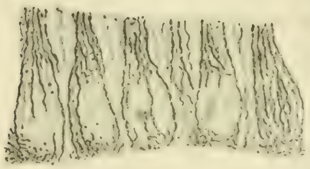

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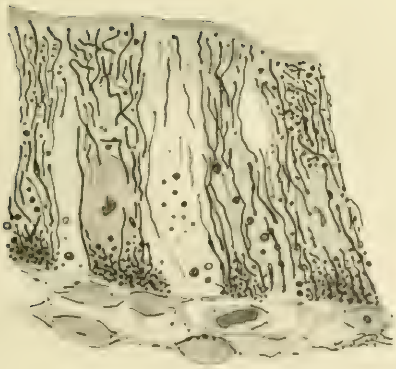

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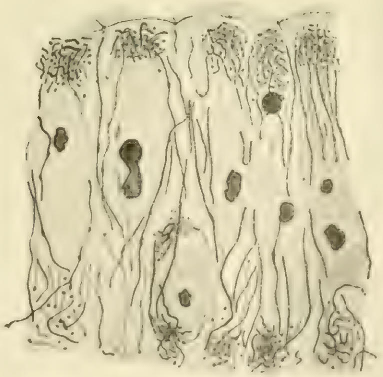

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PLATE 3

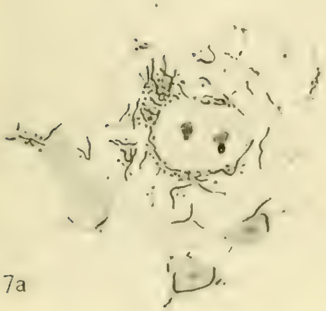
EXPLANATION OF FIGURES

7a Supposed primordial germ-cell with adjacent mesenchyme-cells, in an embryo of Fundulus, nine days old. Fixation: Regaud; stain: fuchsin-methylgreen. Ocular 12.

7b Primordial germ-cell in an embryo of Trout, 24 days old. Fixation: Benda; stain: fuchsin-methylgreen. Ocular 12.

8a Sagittal section of a myotome, the fourteenth from the posterior extremity, in an embryo of Fundulus, three days old. The dorsal edge is turned below, the posterior edge to the left. Fixation: Regaud; stain: fuchsin-methylgreen. Ocular 8.

8b From the same embryo, a myotome, cephalad to the preceding. Same orientation. Ocular 8.



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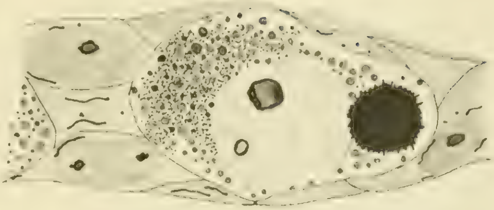

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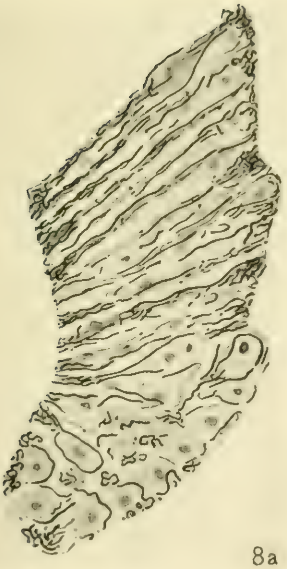

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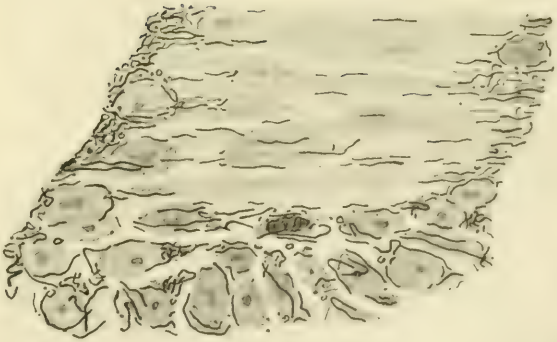

8b

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雞胎羊膜ニ於ケル呼吸血管網ノ位置

ダンチャコフ　述

雞胎ノ發育中、呼吸毛管網ハ始メ羊膜ニ於テ漿膜ノ上皮層上ニ表在性ノ位置ヲトルモ、漸次毛管ハ外皮層內ニ入リ來リ之ヲ横リテ外皮上表ニ網ヲ作リ外皮層ハ毛管網ノ下層ニ位スルニ至ル。

毛管網下層ノ上皮様細胞ハフェルボーンハ之ヲ間胚葉ヨリ起ルト唱ヘシモ、實ハ漿膜外皮ニ屬スルモノナリ。　如何トナレバ實驗的方法ニヨリテ此細胞ノ機能ヲ增進セシメ更ニ進行性變化ヲ惹起セシムルコトヲ得ベク、而シテ此特性ハ外皮系ノ細胞組織ニ特有ノモノナルヲ以テナリ。　又實驗的ニ此組織ヲ刺戟スルコトニ由リテ其ノ反應トシテ增生ヲ來シ、惹テ角化ヲモ起サシムルコトヲ得ベシ。

上述ノ如ク刺戟ニヨリ外皮系ガ特異ナル分離變化（角化）ヲ行フ事實ハ明カニ漿膜外皮ニ起レル變化ガ非恢復性ナルコトヲ示スモノナリ。

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